APHID VECTOR DYNAMICS AND TEMPORAL AND SPATIAL CHARACTERIZATION OF WATERMELON VIRUS EPIDEMICS

By

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To my wife Maria Alejandra who is all I want to have and makes the sense of my life.

To my parents Carlos and Sofia who gave me more than love and affection and whose moral principles and memories are always here, deep in my heart.

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APHID VECTOR DYNAMICS AND TEMPORAL AND SPATIAL CHARACTERIZATION OF WATERMELON VIRUS EPIDEMICS

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Average apparent infection rates of WMV-2 epidemics ranging from 0.193 to 0.225 logit units/day ($r^2 > 0.93$, $p \leq 0.05$) in 1993 and 0.320-0.409 logit units/day ($r^2 > 0.93$, $p \leq 0.05$) in 1994 were found in four experimental watermelon plots (sown 24 and 15 March, respectively) at Leesburg, Florida. The first infected plants were detected on April 30 (1993) and April 25 (1994). Average times of epidemic duration at ≥ 95% incidence were 30 days and 26 days for 1993 and 1994, respectively. In 1993, WMV-2 was detected with ELISA in 97% of 300 plants in one plot on June 7. ZYMV and PRSV-W were not detected. In 1994, WMV-2 was detected with ELISA in 9% of 298 plants in one plot on May 26, whereas ZYMV was detected in 80% of the plants. PRSV-W was not detected in systematic sampling of 80 plants. Fruit yields of single plants or of all plants infected at the same time were directly correlated with the number of days that plants remained healthy. Yield increase rates of 0.146 kg day^{-1} ($r^2 = 0.97$) and 0.140 kg day^{-1} ($r^2 = 0.96$) were found in 1993 and 1994, respectively. Average fruit

sugar content of individual plants was correlated with time of infection in 1994 (r^2 = 0.818) but not in 1993 (r^2 = 0.046). Spatial pattern studies conducted with Gray's two-dimensional distance class indicates presence of random and aggregated patterns of infected plants during development of the epidemics. Disease gradients were described by the non-stationary wave model in 1994 suggesting that local sources played an important role in epidemic build up. In 1993, *Uroleucon pseudambrosiae* (*Up*), *Aphis middletonii* (*Am*), and *A. gossypii* (*Ag*) were the most common aphid species caught in green tile water pan traps (137, 137, and 119, respectively, of the 745 aphids caught). Respective population peaks of these aphids occurred on 14 May, 25 April, and 5 May. In 1994, *U. pseudambrosiae*, *Myzus persicae,* and *A. middletonii* were the most commonly trapped aphids (1390, 113, 97, respectively, of the 2093 aphids caught). Respective population peaks of these aphids occurred on 15 April-4 May, 25 April, and 20 April. Factor analysis, varimax rotated biplot displays, and lag-time regression of factor scores of aphid vectors with incidence change (y) were used to develop two forecasting models. In 1993, the best explanatory model (R^2 = 0.82, Cp-Mallow=2.38) was $\hat{y}$ = 0.09 (*Up*) + 0.10 (*Am*) + 0.011 (*Ag*). In 1994, disease incidence was best predicted with the model $\hat{y}$ = 0.14 (*Up*) + 0.12 (*Am x As*), where *As*= *A. spiraecola* (R^2 = 0.94, Cp-Mallow=1.0). *Am* and *Up* appear to be major vectors during primary and secondary dispersion of WMV-2 and ZYMV in watermelon in Central Florida, respectively. Arena test results indicate that *U. pseudambrosiae* was able to transmit ZYMV (isolate FC3326) in 6 out of 150 plants tested (15 arena tests). This is the first report of this species vectoring ZYMV in watermelon.

CHAPTER 1
INTRODUCTION

Florida leads the nation in watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) production, producing about one-third of the watermelons grown in the United States and ranking third in acreage harvested (Allred and Lucier, 1990; Anonymous, 1994; 1992). Although there has been a decline in the acreage of watermelons harvested in Florida, yield per acre has increased due to development of new varieties, and improvements in production practices and handling (Fig. 1.1) (Allred and Lucier, 1990). Watermelon is the most extensively grown cucurbit crop in Florida with more than 40 thousand acres planted per season from 1990 to 1993, followed by cucumber (*Cucumis sativus* L.) and squash (*Cucurbita pepo* L.) with more than 15 and 12 thousand acres, respectively (Anonymous, 1994-1988). Watermelon is grown in at least 30 of the 67 Florida counties, with the highest harvested acreage in the northern region (Fig. 1.1) (Anonymous, 1994-1988). In the 1991-92 and 1992-93 seasons, Alachua, Gilchrist, Levy, and Suwannee (north); Manatee (central); and Collier (south), had the highest harvested acreage with an average of more than 2.3 thousand (Anonymous, 1994; 1993).

The reduction in acreage planted in the last 10 years reflects several factors including marketability, weather conditions, and pests. Nevertheless, the magnitude of these problems has not been fully evaluated (Anonymous 1992; Allred and Lucier, 1990). Watermelon mosaic virus 2 (WMV-2), papaya ringspot virus type W (PRSV-W), and zucchini yellow mosaic virus (ZYMV) are the most important viruses affecting

watermelon and other cucurbits in Florida (Webb and Linda, 1993; Kucharek and Purcifull, 1989; Purcifull, et al., 1988, 1984a; Adlerz et al., 1983; Adlerz 1978a, 1969).

WMV-2 is the most restrictive production factor in spring crops of watermelon in Central and North Florida, whereas PRSV-W is the predominate virus in both the spring and fall crops in southern regions (Table 2.1). Although ZYMV, the least prevalent virus, is seasonally and regionally more erratic than the other two, fall crops in Central Florida are the most threatened (Webb and Linda, 1993; Purcifull et al., 1988; Adlerz et al., 1983; Table 2.1). These potyviruses are nonpersistently transmitted by at least 42 (WMV-2), 25 (PRSV-W), and 13 (ZYMV) species of aphids on a worldwide basis (Webb et al., 1994; Webb and Kok-Yokomi, 1993; Adlerz, 1987, Tables 2.2-2.5).

Since the first report of a virus-like disease in watermelon (Walker, 1933), extensive research has been conducted to investigate different aspects of PRSV-W, WMV-2, and ZYMV in Florida. Host range and serological techniques, including DAS-ELISA and sodium dodecyl sulfate (SDS)-immunodiffusion with antisera to the coat protein have been used to identify, detect, and survey for these viruses (Webb and Linda, 1994; Baker et al., 1991; Purcifull et al., 1988, 1984a, 1981; Alderz et al 1983; Purcifull and Hiebert, 1979; Adlerz, 1969; Webb et al., 1965b; Webb and Scott, 1965; Lindberg et al., 1956; Anderson, 1954, 1952, 1951). Recently, serological tests with nonstructural proteins (Purcifull and Hiebert, 1992; Baker and Purcifull, 1990; de Mejia et al., 1985), were utilized for characterization of some of these viruses at the strain level (Wisler et al., 1995)

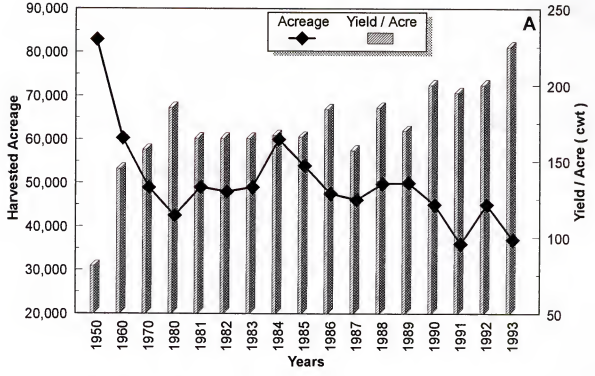
Abundance, composition, and transmission by different aphid species, as well as the effect of color and trap type on the numbers of aphids collected, have also been studied in Florida (Webb and Kok-Yokomi, 1993; Webb, 1992; Adlerz, 1987; 1974a). Integration of this information with reports of WMV-2, PRSV-W, and ZYMV vectors on a worldwide basis (Tables 2.2-2-4) has led to the determination that 15 (WMV-2), 13 (PRSV-W), and 8 (ZYMV) of these vectors are present in Central Florida. Among these,

Aphis spiraecola, *A. middletonii*, and possibly *Myzus persicae* have been considered the most important vectors driving spring epidemics (Adlerz, 1987; 1978b; 1974a). Whether or not these species are quantitatively related to changes in disease incidence still needs to be demonstrated.

Adlerz (1972a, 1972b) suggested that two cucurbitaceous weeds, *Melothria pendula* and *Momordica charantia*, were the most likely sources of PRSV-W in South Florida. The epidemiological implications of these weeds have been studied, and possible control measures have been generated based on that information (Kucharek and Purcifull, 1989; Adlerz 1972a, 1972b). Weed hosts that would allow WMV-2 and ZYMV to survive the winter in Central and North Florida have not been found. Wind effects on spread of PRSV-W from local sources to watermelon have also been documented (Alderz, 1974b); however, the impact of this factor as a regional event has not been addressed.

The control of WMV-2, PRSV-W, and ZYMV has been attempted with applications of JMS stylet oil and/or endosulfan (Webb and Linda, 1993) and with mulches of aluminum foil and white polyethylene (Adlerz and Everett, 1968). Promising results were obtained under conditions of low disease intensity. However, specific conditions for changes of disease intensity through the season are not fully understood.

To continue the basic research conducted with WMV-2, PRSV- W, and ZYMV in Florida, this research was designed from the perspective of quantitative epidemiology. The overall scope was to determine quantitatively the epidemiological factors involved in the spread of these potyviruses in watermelon in North Central Florida. The ultimate goal was to provide an understanding of the development process of epidemics at the field level and to provide practical information for use in disease management. To address this goal, integrated studies were carried out on the following epidemiological aspects: plant-virus-vector system, yield loss modeling, and virus disease incidence as it relates to spatial patterns, temporal spread, and forecasting.



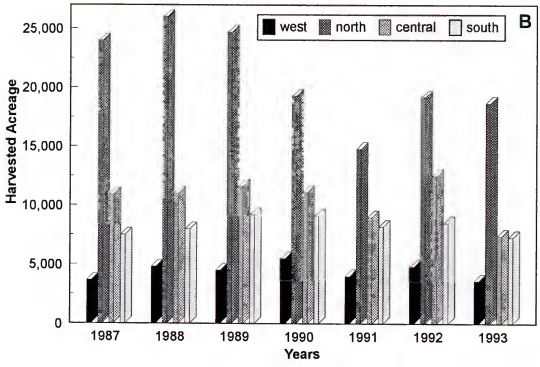


Figure 1.1. **A.** Florida watermelon acreage harvested (■—■) and yield per acre (bars). **B.** Acreage of watermelon harvested in the West, North,Central, and South Florida areas. Data from: Florida Agricultural Statistics, Vegetable Summary 1986 -1993, and from Allred, A. J. and Lucier, G. 1990. The U. S. Watermelon Industry. USDA. Report No. AGES 9015.

CHAPTER 2
LITERATURE REVIEW

Potyviruses in Watermelon in Florida

Watermelon mosaic virus (WMV-2), papaya ringspot virus type W (PRSV-W), and zucchini yellow mosaic virus (ZYMV) cause the most important viral diseases in watermelon (*Citrullus lanatus*) in Florida (Webb and Linda, 1993; Purcifull et al., 1988; Purcifull et al., 1984a; Adlerz et al., 1983; Adlerz, 1978a; Adlerz, 1974b; Adlerz, 1972a; Adlerz, 1972b; Adlerz, 1969; Adlerz, 1968) and in other parts of the world (Dahal, 1992; Lecoq et al., 1991b; Wang et al., 1991; Lovisolo, 1980).

General Characteristics

WMV-2, PRSV-W, and ZYMV are members of the *Potyvirus* genus of the Potyviridae family according to the International Committee on Taxonomy of Viruses (ICTV) (Shukla et al., 1994). These viruses are flexuous rod-shaped particles about 11-15 nm in diameter and 750 nm in length with a single strand of RNA. Their genome encodes eight proteins which are translated as polyproteins in a single open reading frame (Shukla et al., 1994; Brunt, 1992; Brunt et al., 1990). Characteristically, these potyviruses induce cytoplasmic inclusions (pinwheels) in infected hosts (Edwardson and Christie, 1991). These viruses are transmitted nonpersistently by at least 42 (WMV-2), 25 (PRSV-W), and 13 (ZYMV) species of aphids (Webb and Kok-Yokomi, 1993; Castle et al., 1992; Adlerz, 1987; Yamamoto et al., 1982; Karl and Schmelzer, 1971; Tables 2.2-2.4) and are easily transmitted by sap inoculation (Purcifull et al., 1984b; Purcifull et al., 1984c; Lisa and Lecoq, 1984). Those that infect cucurbits have

not conclusively been shown to be seedborne (Orosco-Santos et al., 1994; Robinson et al., 1993; Purcifull et al., 1984c; Lecoq et al., 1981).

History and Host Range of WMV-2, ZYMV, PRSV-W

The first report of a virus-like disease in watermelon (*C. lanatus*) in Florida was in 1933 (Walker, 1933). In this report, up to one percent of aggregated to randomly infected plants were found in four adjacent fields located in Central Florida. Symptoms exhibited by plants included a "petunia-like" appearance of new shoots, leaf proliferation, stunting, diffuse yellow mottling, severe leaf and flower deformation, and either no fruit set or malformed fruit. No test was conducted to determine the causal agent. In 1951, Anderson found a virus isolate in squash in Central Florida which caused symptoms similar to those described by Walker (1933) and suggested the name of "southern squash mosaic" (Anderson, 1951). In further studies, this virus was considered to be a member of the watermelon mosaic complex (Anderson, 1952; 1954) which included two viruses: "yellow watermelon mosaic" and "watermelon mosaic." The former virus induced symptoms similar to those originally described by Walker (1933) and caused symptomatic systemic infection in *Luffa acutangula* (Anderson, 1954), a characteristic of most PRSV-W isolates (Adlerz et al., 1983). A later investigation, conducted by Lindberg and coworkers with 13 cucurbit virus isolates from several areas of the United States, classified the two viruses of Anderson as strains of "melon mosaic virus" (Lindberg et al., 1956). Serological tests and electron microscopy were used for the first time to characterize these isolates.

In 1965, a more definitive characterization of the watermelon complex was provided by Webb and Scott using host range, physical properties, serology, and cross protection of 10 isolates infecting cucurbits in the southern and western United States. They suggested the validity of the separation of the "melon mosaic virus" into two

distinct viruses and referred to them as WMV-1 and WMV-2 (Webb and Scott, 1965a, b). In 1969, however, these two viruses were again considered strains of the same virus because a close serological relationship was found between them (Milne and Grogan, 1969). It was not until 1979 that these strains were finally recognized as independent viruses. Moreover, WMV-1 and papaya ringspot virus were found to be serologically related (Purcifull and Hiebert, 1979). Currently, WMV-1 is considered a strain of papaya ringspot virus and is formally described as PRSV-W, whereas WMV-2 still maintains its original name (Purcifull et al., 1984b, 1984c).

WMV-2 has the broadest host range among the potyviruses in watermelon, infecting 178 species in 79 genera of 27 families (Edwardson and Christie, 1991). Of all species reported, 50 are cucurbits, of which only watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus*), cantaloupe (*Cucumis melo*), and squash (*Cucurbita pepo*) are grown extensively in Florida. Forty-five of the suscepts are legumes, of which only snap bean (*Phaseolus vulgaris*) is a relatively important crop in Florida.

PRSV-W naturally infects only cucurbits (39 species in 12 genera), with squash, watermelon, cucumber, and cantaloupe among the economically important crops. Experimentally, PRSV-W causes local lesions in *Chenopodium amaranticolor* and *C. quinoa* (Chenopodiaceae) (Edwardson and Christie, 1991; Purcifull et al., 1984b).

ZYMV was originally observed and described from zucchini (*C. pepo*) in northern Italy in 1973 and from muskmelon (*C. melo*) in southern France in 1979 by Lisa et al. (1981) and Lecoq et al. (1981), respectively. A further description of this virus was given by Lisa and Lecoq (1984). This virus was first reported in the United States in 1981 infecting squash in Florida (*C.pepo*) (Adlerz et al., 1983; Purcifull et al., 1984a). ZYMV was found in the same crop in northern Connecticut in 1982 (Provvidenti et al., 1984), and in several cucurbit crops in western New York and central California in 1983 (Provvidenti et al., 1984), in southern California (Nameth et al., 1985) and New Jersey in 1985 (Davis and Mizuki, 1987), and in Hawaii in 1988 (Ullman et al.,

1991). ZYMV infects mostly cucurbits (29 species in 7 genera) including cucumber, squash, watermelon, and cantaloupe. Another ten families with 15 genera comprising 17 species have also been reported to be infected experimentally or naturally by this virus (Edwardson and Christie, 1991; Adlerz et al., 1983; Lecoq et al.,1981).

Diagnosis of WMV-2, ZYMV and PRSV-W

Because of the great biological variability of potyviruses and the frequency of mixed infections, symptoms are not a reliable approach for diagnosis of diseases caused by WMV-2, ZYMV, and PRSV-W in watermelon and other cucurbit crops (Lisa and Lecoq, 1984). Moreover, at least other two viruses, cucumber mosaic virus (CMV) and squash mosaic virus (SMV), have also been detected in naturally infected cucurbits in Florida (Purcifull et al., 1988, Kucharek and Purcifull, 1989). Worldwide, at least 23 viruses are known to infect cucurbits under natural or experimental conditions (Brunt et al., 1990). Among those that have been associated with epidemics in the United States are curly top virus (Nelson and Tuttle, 1969), lettuce infectious yellows, squash leaf curl virus, and melon leaf curl virus (Perring et al., 1989; Duffus et al., 1986; 1985; Dodds et al., 1984).

Serological techniques, including double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and sodium dodecyl sulfate (SDS)-immunodiffusion with polyclonal and/or monoclonal antisera to the coat protein have been successfully used for characterization of viruses or strains, regional surveys, and for epidemiological studies of potyviruses in Florida (Webb and Linda, 1993; Baker et al., 1991; Purcifull et al., 1981, 1984a, 1988; Quiot-Douine et al., 1986; Adlerz et al., 1983; Purcifull and Hiebert, 1979). However, because of the high biological variability of potyviruses (Lecoq and Purcifull, 1992), and problems with identifying some viruses at the strain level (Wisler, 1992), antisera to nonstructural proteins have been prepared

for identification and classification of potyviruses (Purcifull and Hiebert, 1992). These nonstructural proteins include the cylindrical inclusion, protease (small nuclear inclusion protein), replicase (large nuclear inclusion protein), helper component-protease or amorphous inclusion protein, P1 protein, and P3 protein (Purcifull and Hiebert, 1992; Wisler, 1992; Baker and Purcifull, 1990; de Mejia et al., 1985). Wisler et al. (1995) showed that the P1 protein was useful for detecting variability of ZYMV isolates, including three isolates from Florida.

Whatever the criteria used for identification of potyviruses in epidemiological studies, one should take into consideration that the technique selected must remain simple to be applied to a large number of isolates or field samples and entail use of rather simple equipment (Lecoq and Purcifull, 1992).

Occurrence and Distribution of WMV-2, PRSV-W, and ZYMV in Florida

Distribution of WMV-2. Although the first indication of a virus-like disease in watermelon was reported in 1933 (Walker, 1933), it was not until 1965 that the first survey of viruses was conducted in the southern and western regions of the United States, including Florida (Webb et al., 1965b). In this survey, WMV-2 was found in both south and Central Florida. Interestingly, this is the only survey that has reported WMV-2 in South Florida. In recent surveys, WMV-2 has been detected in watermelon as well as in the other major cucurbit crops in both North and Central Florida but not in the southern region (Purcifull et al., 1988, 1984a; Adlerz et al., 1983) (Table 2.1). Although a survey conducted in 1981-83 in eight counties determined that WMV-2 was present in similar proportions in spring and fall in both North and Central Florida (Adlerz et al., 1983), a later, more extensive survey performed in 1987 and 1988 in 21 counties showed WMV-2 to be more prevalent during the spring (January-July) than the fall season (August-November) for these regions (Purcifull et al., 1988) (Table 2.1).

Table 2.1. Distribution and occurrence of watermelon mosaic virus type 2 (WMV-2), papaya ringspot virus (PRSV-W), and zucchini yellow mosaic virus (ZYMV) infecting various cucurbit crops in Florida.

Year [1]	WMV-2 [2]	PRSV-W	ZYMV	W + P	W+ Z	P + Z	Reference
North Florida / Spring [3]							
1981-1983	17 / 21	0 / 21	4 / 21	0 / 21	0/21	0 / 21	Adlerz et al., 1983 [4]
1987-1988	52 / 97	3 / 97	0 / 97	-	-	-	Purcifull et al., 1988 [5]
North Florida / Fall							
1981-1983	19 / 40	9 / 40	2 / 40	8 / 40	0/40	2 / 40	Adlerz et al., 1983
1987-1988	33 / 102	46 / 102	7 / 102	-	-	-	Purcifull et al., 1988
North Florida / Undetermined							
1969	0 / 10	10 / 10	-	-	-	-	Adlerz, 1969 [6]
Central Florida / Spring							
1964	4 / 4	0 / 4	-	-	-	-	Webb et al.,1965 [7]
1981-1983	15 / 30	1 / 30	11 / 30	0 / 30	1/30	2 / 30	Adlerz et al., 1983
1987-1988	101 / 111	4 / 111	13 / 111	-	-	-	Purcifull et al., 1988
1989	471 / 480	2 / 480	0 / 480	-	-	-	Webb and Linda,1993 [8]
1990	473 / 480	12 / 480	76 / 480	-	-	-	Webb and Linda, 1993
Central Florida / Fall							
1981-1983	14 / 93	19 / 93	54 / 93	1 / 93	0/93	5 / 93	Adlerz et al., 1983
1987-1988	18 / 53	49 / 53	25 / 53	-	-	-	Purcifull et al., 1988
1988	0 / 160	24 / 160	0 / 160	136 / 160	0/640	0/ 640	Webb and Linda, 1993
Central Florida / Undetermined							
1965-1969	48 / 57	9 / 57	-	-	-	-	Adlerz, 1969
South Florida / Spring							
1981-1983	0 / 60	45 / 60	11 / 60	0 / 60	0/60	4 / 60	Adlerz et al., 1983
1987-1988	0 / 112	83 / 112	4 / 112	-	-	-	Purcifull et al., 1988
1962-1963	11/ 20	8 / 20	-	-	-	-	Webb et al., 1965
1964-1965	4 / 28	26 / 28	-	-	-	-	Webb et al.,1965
South Florida / Fall							
1981-1983	0 / 43	41 / 43	0 / 43	0 / 43	0/43	2 / 43	Adlerz et al., 1983
1987-1988	0 / 74	60 / 74	2 / 74	-	-	-	Purcifull et al., 1988
1962	6 / 6	0 / 6	-	-	-	-	Webb et al.,1965
South Florida / Undetermined							
1966-1969	0 / 44	44 / 44	-	-	-	-	Adlerz, 1969

[1] Year of sampling.

[2] WMV-2= watermelon mosaic virus, PRSV-W= papaya ringspot virus, ZYMV= zucchini yellow mosaic, W+P= mixed infection of WMV-2+PRSV-W, W+Z=WMV-2+ZYMV, P+Z=PRSV-W+ZYMV.

[3] Florida region and growth season. Undetermined = undetermined growth season.

[4] Non-random sampling; viruses diagnosis by host range and SDS immunodifusion test (antiserum -As- 862 / PRSV-W FL, As 868 / WMV-2FL, AS 1028 ZYMV-FL).

[5] Usually non-random sampling; diagnosis by SDS immunodifusion test (As 1125/ PRSV-W, As 1117 and 1134 / WMV-2, AS 1133 ZYMV).

[6] Sampling method not indicated; diagnosis by host range.

[7] Non-random sampling; virus diagnosis by host range and serology for PRSV-W .

[8] 1988, Non-random sampling (27-31 October); 1989 (21 June) and 1990 (14 June) random sampling. Diagnosis by ELISA for all years (antiserum not indicated).

The spring incidence of this virus was also confirmed in random samplings conducted in 1989 and 1990 in experimental fields of watermelon in North Central Florida (Webb and Linda, 1993). In this sampling, 471 out of 480, and 473 out of 480 plants were serologically positive for WMV-2 in 1989 and 1990, respectively (Table 2.1). These results clearly show that this virus is ecologically well adapted to the conditions of the area and therefore, it may constitute a limiting factor for watermelon production in Central Florida.

Distribution of PRSV-W. PRSV-W is one of the most prevalent viruses in South Florida in both spring and fall but appears to be almost restricted to the fall crops in North and Central Florida (Webb and Linda, 1993; Purcifull et al., 1988; Purcifull et al., 1984a; Adlerz et al., 1983; Adlerz, 1969; Webb et al., 1965b). Whereas this virus is endemic and limits squash production in southeastern Florida (Adlerz et al., 1983), it has erratic and low incidence in central and northern cucurbit production areas (Table 2.1).

In Central Florida, for example, random sampling of experimental fields of watermelon showed PRSV-W in 24 out of 160 plants in the fall of 1988, and in 2 out of 480 and 12 out of 480 plants in the springs of 1989 and 1990, respectively (Webb and Linda, 1993) (Table 2.1). In North Florida, surveys conducted in a total of 10 counties during in 1987-1988 detected PRSV-W in 46 out of 102 plants in the fall, and in 3 out of 97 cucurbitaceous plants in the spring (Purcifull et al., 1988).

Distribution of ZYMV. ZYMV was first reported in Florida in 1981, and it was present from November 1981 to September 1983 in eight out of nine counties sampled in Florida (Adlerz et al., 1983) (Table 2.1). In general, this virus was more prevalent in Central Florida counties than other regions of the state, particularly in fall crops. In 1987 and 1988, ZYMV was detected in 11 of 21 counties sampled (Purcifull et al., 1988). Among those counties where the virus was found in 1987 or 1988, one did not

have ZYMV in 1981 (Adlerz et al., 1983) and five were counties sampled for the first time. Again the virus was more prevalent in the fall crops of Central Florida.

Seasonal incidence of ZYMV appears to be more erratic than the other potyviruses affecting watermelon. For example, by the end of the spring season of 1989 (21 June) no ZYMV was serologically detected in 480 samples randomly selected from experimental fields of watermelon in Central Florida; in contrast, in the spring of 1990 (14 June) the virus was found in 76 out of 480 randomly chosen plants (Webb and Linda, 1993).

Although no information has been provided in some studies regarding mixed infections (Purcifull et al., 1988), mixed infections of ZYMV with PRSV-W have been reported more frequently than with WMV-2 in Florida (Adlerz et al., 1983) (Table 2.1). The opposite situation was observed in fall crops of cucurbits in New Jersey by Davis and Mizuki (1987). In that study, ZYMV was highly aggressive in squash and appeared to have a competitive advantage over PRSV-W and WMV-2 in mixed infections, both in the field and under experimental conditions. For that reason, they suggested performing ELISA directly on field samples to detect virus infection rather than with plants rub-inoculated with sap from field samples.

Aphid Vectors of WMV-2, PRSV-W, and ZYMV

Watermelon mosaic virus-2 (WMV-2), papaya ringspot virus type W (PRSV-W), and zucchini yellow mosaic (ZYMV), have been reported to be transmitted by aphids in a nonpersistent manner and with a low degree of specificity (Berger, 1992; other references in Tables 2.2-2.4). These viruses have been experimentally or are naturally transmitted by at least 42 (WMV-2), 25 (PRSV-P), and 13 (ZYMV) aphid species, respectively (Tables 2.2-2.4).

Table 2.2. Aphid species known worldwide to transmit watermelon mosaic virus 2 (WMV-2) to a cucurbit host under natural or experimental conditions.

Aphid species	Common name [1]	Transmission [2]	Reference
Acyrthosiphon kondoi		4/132(alate-lab.)	Castle et al., 1992
		3/19 (natural infect.)[3]	Castle et al., 1992
A. lactucae		-	Edwardson & Christie,1991
A. pisum	pea aphid	10/64 (alate-lab.)	Castle et al., 1992
		10/171(natural infect.)	Castle et al., 1992
		2/7	Yamamoto et al., 1982
		6/24	Karl & Schmelzer, 1971
Aphis middletonii	erigeron root aphid	5/28 (alate-field)	Adlerz, 1987
		15/404	Adlerz, 1987
A. craccivora	cowpea aphid	2/25	Yamamoto et al., 1982
		35/36	Karl & Schmelzer, 1971
A. fabae	bean aphid	13/24	Karl & Schmelzer, 1971
A. gossypii	cotton aphid	24/50	Yamamoto et al., 1982
		23/144(alate-lab)	Castle et al., 1992
		6/20	Coudriet, 1962
A. illinoisensis	grapevine aphid	3/11 (natural infect.)	Adlerz, 1987
A. nerii	oleander aphid	5/10	Yamamoto et al., 1982
A. spiraecola (=A. citricola)	spirea aphid	9/141(alate-field)	Adlerz, 1987
		21/573(natural infect.)	Adlerz, 1987
		3/44 (alate-lab)	Castle et al., 1992
A. frangulae gossypii		48/48	Karl & Schmelzer, 1971
A. glycines		7/20	Yamamoto et al., 1982
A. nasturtii	buckthorn aphid	18/24	Karl & Schmelzer, 1971
A. sambuci		6/24	Karl & Schmelzer, 1971
Aulacorthum circumflexus		12/24	Karl & Schmelzer, 1971
A. magnoliae		10/45	Yamamoto et al., 1982
A. nipponicum		6/10	Yamamoto et al., 1982
A. solani	foxglove aphid	3/10	Yamamoto et al., 1982
		28/36	Karl & Schmelzer, 1971
Brachycaudus cardui	thistle aphid	8/24	Karl & Schmelzer, 1971
Cerosipha gossypii			Edwardson & Christie,1991
Cryptomyzus ribis	currant aphid	9/24	Karl & Schmelzer, 1971
Dysaphis crataegi		15/24	Karl & Schmelzer, 1971
Hyperomyzus sp[4]		4/20	De Sa and Kitajima, 1991
Hyalopterus pruni	mealy plum aphid	5/24	Karl & Schmelzer, 1971
Hysteroneura setariae	rusty plum aphid	1/20	Coudriet, 1962
Macrosiphum barri		6/19	Coudriet, 1962
M. euphorbiae	potato aphid	1/10	Yamamoto et al., 1982
		21/24	Karl & Schmelzer, 1971
M. pisi		1/20	Coudriet, 1962
Macrosiphoniella sanborni		3/32	Karl & Schmelzer, 1971
Myzus persicae	green peach aphid	4/26 (alate-field)	Adlerz, 1987
		9/10	Yamamoto et al., 1982
		31/168(alate-lab.)	Castle et al., 1992
		28/175(natural infect.)	Castle et al., 1992
		17/20	De Sa and Kitajima, 1991
		57/60	Karl & Schmelzer, 1971
		19/20	Coudriet, 1962
M. cerasi	black cherry aphid	1/24	Karl & Schmelzer, 1971
Phorodon humuli	hop aphid	21/24	Karl & Schmelzer, 1971

Table 2.2.---continuated

Aphid species	Common name [1]	Transmission [2]	Reference
P. humuli janonensis		5/9	Yamamoto et al., 1982
Rhodobium porosum		5/10	Yamamoto et al., 1982
Rhopalosiphum maidis	corn leaf aphid	2/20	Coudriet, 1962
R. padi	bird cherry-oat aphid	1/10	Yamamoto et al., 1982
		2/24	Karl & Schmelzer, 1971
		1/23 (natural infect.)	Castle et al., 1992
Semiaphis dauci		6/24	Karl & Schmelzer, 1971
Toxoptera citricida	brown citrus aphid	6/28	Yamamoto et al., 1982
Uroleucon ambrosiae		13/20	De Sa and Kitajima, 1991
U. formosanum		2/10	Yamamoto et al., 1982
U. pseudambrosiae		53/450 [4]	Webb & Kok-Yokomi,1993
		23% ± 12 SD [5]	
U. gobonis		3/15	Yamamoto et al., 1982

[1]Common names as listed in: Common Names of Insects & Related Organisms, edited by the Entomological Society of America, 1989.
[2]Number of infected plants / number of plants accessed. Tests conducted under controlled conditions with apterae, nymphs, or alates reared in the laboratory, or collected in the field (alate-field) and given immediate access to a virus source plant. De Sa and Kitajima (1991) and Coudriet (1962) used 10 aphids per test plant.
[3] Alate aphids collected in the field and given immediate access to a test plant.
[4]Accumulated data of 12 controlled tests conducted with three acquisition and two starvation times.
[5]Average percentage of infected plants in 15 arena tests.
[6]This species was not fully identified. Two possibilities were mentioned: *H. carduellinus* and *H. lactucae*.

Table 2.3 Aphid species known worldwide to transmit papaya ringspot virus type W (PRSV-W) to a cucurbit host under natural or experimental conditions.

Aphid species	Common name [1]	Transmission [2]	Reference
Acyrthosiphon pisum	pea aphid	6/24	Karl & Schmelzer, 1971
Aphis craccivora	cowpea aphid	10/24	Karl & Schmelzer, 1971
A. fabae	bean aphid	9/24	Karl & Schmelzer, 1971
A. frangulae gossypii		9/24	Karl & Schmelzer, 1971
A. gossypii	cotton aphid	16/20 ,18/20[3]	Labonne et al., 1992
		26/30	Tewari, 1976
A. nasturtii	buckthorn aphid	11/26	Karl & Schmelzer, 1971
A. nerii	oleander aphid	15/30	Tewari, 1976
A. sambuci		11/24	Karl & Schmelzer, 1971
A. spiraecola (=A. citricola)	spirea aphid	-	Edwardson & Christie,1991
Aulacorthum circumflexus		3/40	Karl & Schmelzer, 1971
A. solani	foxglove aphid	16/24	Karl & Schmelzer, 1971
Brachycaudus cardui	thistle aphid	4/24	Karl & Schmelzer, 1971
B. helichrysi		1/40	Karl & Schmelzer, 1971
Cavariella aegopodii		7/48	Karl & Schmelzer, 1971
Cryptomyzus ribis	currant aphid	1/24	Karl & Schmelzer, 1971
Dysaphis crataegi		3/24	Karl & Schmelzer, 1971
Hyalopterus pruni	mealy plum aphid	9/24	Karl & Schmelzer, 1971
Lipaphis erysimi (=Hyadaphis pseudobrassicae)		-	Adlerz, 1974
Lipaphis pseudobrassicae		18/30	Tewari, 1976
Macrosiphum euphorbiae	potato aphid	10/24	Karl & Schmelzer, 1971

Table 2.3---continuated

Aphid species	Common name [1]	Transmission [2]	Reference
Myzus persicae	green peach aphid	43/48	Karl & Schmelzer, 1971
		24/30	Tewarl, 1976
M. cerasl	black cherry aphid	2/24	Karl & Schmelzer, 1971
Phorodon humuli	hop aphid	11/24	Karl & Schmelzer, 1971
Sitobion rosaformis		-	Edwardson & Christle,1991
Uroleucon pseudambrosiae		41% ± 22 SD [4] (arena test)	Webb & Kok-Yokomi, 1993

[1] Common names as listed in: Common Names of Insects & Related Organisms, edited by the Entomological Society of America, 1989.
[2] Number of infected plants / number of plants accessed. Tests conducted under controlled conditions with apterae or nymphs. Tewarl (1976) used five aphids per test plant.
[3] Average values of 16 and 9 replicates, respectively.
[4] Average percentage of infected plants in 10 arena tests.

Table 2.4 Aphid species known worldwide to transmit zucchini yellow mosaic virus (ZYMV) to a cucurbit host under natural or experimental conditions.

Aphid species	Common name [1]	Transmission [2]	Reference
Acyrthosiphon pisum	pea aphid	1/2 (alate-field)	Adlerz, 1987
		2/56 (alate-lab.)	Castle et al., 1992
		1/171 (natural)[3]	Castle et al., 1992
A. kondoi		5/49 (alate-field)	Castle et al., 1992
Aphis craccivora	cowpea aphid	1/3 (alate-field)	Adlerz, 1987
A. gossypii	cotton aphid	92% 72% [3]	Lecoq et al., 1981
		31/88 (alate-lab.)	Castle et al., 1992
A. middletonii		11/42 (alate-field)	Adlerz, 1987
A. spiraecola (=A. citricola)	spirea aphid	7/25	Purcifull et al., 1984a
		19/119 (alate-field)	Adlerz, 1987
Brachycaudus rumexicolons		-	Castle, 1989 (clt. Perring et al., 1989.
Lipaphis erysimi		1/4 (alate-field)	Adlerz, 1987
Macrosiphum euphorbiae	potato aphid	-	Edwardson &Christle,1991
Myzus persicae	green peach aphid	72% 84% [4]	Lecoq et al., 1981
		5/25	Purcufull, 1984a
		1/47 (natural)	Adlerz, 1987
		6/23 (alate-field)	Adlerz, 1987
		52/128 (alate-lab.)	Castle et al., 1992
		1/175 (natural)	Castle et al., 1992
Uroleucon sp.		1/9 (alate-field)	Adlerz, 1987
Uroleucon ambrosiae		-	Orosco-Santos et al., 1994

[1] Common names as listed in: Common Names of Insects & Related Organisms, edited by the Entomological Society of America, 1989.
[2] Number of infected plants / number of plants accessed. Tests conducted under controlled conditions with apterae, nymphs, or alates reared in the laboratory or collected in the field (alate-field) and given immediate access to a virus source plant.
[3] Alate aphids collected in the field and given immediate access to a test plant.
[4] Percentage of infected plants in multiple access tests.

Studies of Transmissibility.

Although most studies of transmissibility of WMV-2, PRSV-W, and ZYMV have been conducted as controlled access tests with a single aptera or nymph given access to individual plants (single-aphid-transfer method) (Purcifull et al., 1984; Karl and Schmelzer, 1971), other experimental conditions have been employed. For example, multiple-aphid-acquisition was used by Yamamoto et al. (1982) to study transmissibility of WMV-2, and the multiple-aphid-transfer method was used by Lecoq et al. (1981) to test ZYMV. In other studies, Adlerz (1987) and Castle et al. (1992) used alate aphids directly trapped in the field in controlled tests for WMV-2 and ZYMV, and Webb and Kok-Yokomi (1993) performed arena tests in addition to some single-aphid-transfer tests for PRSV-W and WMV-2.

Thus, comparisons among virus-vector systems regarding efficiency of transmission would not be valid due to variation in experimental conditions. Similarly, results of tests with a single isolate of the test virus and single clones of the test aphid species should be interpreted cautiously in relation to events occurring under natural conditions (Castle et al., 1992).

In plant virus epidemiology, specific studies about relative transmission rates of aphid vectors are essential for simulation models (Plumb et al., 1986; Sigvald, 1986; Ruesink et al., 1986).

For other approaches to modeling virus epidemics (i.e., stochastic models) regional knowledge of the species composition of aphid vectors based on local or worldwide reports is fundamental (Madden et al., 1983; Marcus and Raccah, 1986; Raccah et al.,1988; Mora-Aguilera et al., 1993b). In such cases, the average vector propensity is usually estimated through statistical models rather than experimentally. Yet, success of these approaches depends upon information about known vectors of the plant-virus system under study.

Efficiency of Transmission and Epidemics

Potyviruses are, in general, efficiently transmitted by aphids (Berger, 1992). In an interesting study (Pirone and Thornbury, 1988), it was shown that acquisition of 15 to 456 particles of tobacco etch or tobacco vein mottling virus was required by *Myzus persicae* to be able to successfully infect healthy tobacco seedlings. No correlation between the number of particles acquired and the ability to transmit was found in this study. Thus, a few sources of inoculum nearby or within a field could potentially cause the development of an epidemic (Hander et al., 1993; Castle, 1992; Gray et al., 1986; Nelson and Tuttle, 1969).

Aphid transmissibility can be reduced or lost due to a deficiency in the viral coat protein or in the production of biologically inactive helper component, which is a viral gene product necessary for transmission to occur (Huet et al., 1994; Granier et al., 1993; Gal-On et al., 1992; Lecoq et al., 1991a; Lecoq, 1986). The age of an infection may be another cause for reduction of transmissibility (Castle, 1992), perhaps due to a decline in virus titer (Romanow et al., 1986).

Loss of aphid transmissibility has been reported for isolates of bean yellow mosaic (Evans and Zettler, 1970; Swenson, 1957, 1964), peanut mottle (Paguio and Kuhn, 1976), potato virus C (Kassanis and Govier, 1971), sugarcane mosaic (Koike, 1979), tobacco etch (Simons, 1976), turnip mosaic (Pound et al. 1932), and zucchini yellow mosaic viruses (Lecoq and Purcifull, 1992).

Because loss of transmissibility has generally been observed after successive mechanical transfers of a virus to a susceptible host under controlled conditions (Lecoq and Purcifull, 1992; Koike, 1979; Simons, 1976; Swenson, 1957), one can speculate that, if this condition occurs at the field level, the probabilities of survival of such strains are relatively small. Thus, these strains must have little impact on the development of epidemics driven by aphid vectors. A different scenario may occur with mixed

infections of related viruses or strains of the same virus. It has been reported that a helper-deficient ZYMV isolate (ZYMV-WK) may be transmitted efficiently by aphids if coinfected with WMV-2 (Lecoq et al., 1991b). Similar results have been reported with a nonaphid-transmissible strain of tobacco etch virus in mixed infection with an aphid-transmissible potato virus Y isolate, but not with an aphid-transmitted watermelon mosaic virus isolate (Simons, 1976). Analogously, a necrotic strain (N) of peanut mottle virus (PMV) was only transmitted when mixed with the M strain of the same virus (Paguio and Kuhn, 1976).

In summary, aphid transmissibility of ZYMV, WMV-2, and PRSV-W, and potyviruses in general, is a complex, interactive process involving genetics of the virus, presence of other potyviruses, density of vector species, etc. In Florida, the diverse species composition of aphid vectors of ZYMV, PRSV-W, and WMV-2 (Webb et al., 1994; Webb and Kok-Yokomi, 1993; Adlerz, 1987), provides an important requirement for epidemics to occur. However, the significance of this component has to be considered in a broader context, where the host, virus, and environment may play important roles (Berger and Ferriss, 1989; Irwin and Ruesink, 1986).

WMV-2, PRSV-W, and ZYMV Transmission Studies: Emphasis on Florida

Several studies have been carried out to determine the species composition of alate aphids present in cucurbit production areas and to determine the transmissibility of WMV-2, ZYMV, and PRSV-W by their aphid vectors in North Central Florida. An extensive study was conducted with naturally infectious alates caught in screen, suction, and yellow water pan traps during spring epidemics in cucurbit plantings. In three years, only 4 out of 40 species transmitted WMV-2, ZYMV, or both, but none transmitted PRSV-W (Adlerz, 1987). Of these, 92% of all viruliferous aphids were either *Aphis spiraecola* (52%) or *A. middletonii* (40%). Both of these aphids transmitted

WMV-2 and ZYMV. The remaining 8% of all vectors were *Myzus persicae*, transmitting ZYMV, and *A. illinoisensis*, transmitting WMV-2. The first viruliferous aphid trapped in this study was *A. middletonii*, confirming results from an earlier study (Adlerz, 1978b) in which this species was found to be the only vector trapped on most days during a severe epidemic outbreak. This aphid transmitted WMV-2 every time a test was conducted with naturally infectious alates trapped in fields with a disease incidence of at least 4%. Adlerz (1987) concluded that only two or three aphid species (i.e., *A. spiraecola*, *A. middletonii*, and possibly *M. persicae*) could be important virus vectors in spring epidemics in North Central Florida.

In a three-year study in southern California, Castle et al. (1992) found natural transmission of WMV-2 and ZYMV by *Myzus persicae* (29 out of 8206 individuals tested) and *Acyrthosiphon pisum* (11 out of 2430) and of WMV-2 by *A. kondoi* (3 out of 3759) and *Rhopalosiphum padi* (1/1113). Because Castle and coworkers rarely found naturally inoculative aphids (44 out of 18,837 aphid tested), they concluded that primary infection from external sources occurs with low frequency. Castle et al. (1992) attributed the build up of virus epidemics to secondary dispersion within and adjacent to muskmelon fields, *Myzus persicae* and *Aphis gossypii* being the most efficient vectors of WMV-2 and ZYMV.

In another study (Castle, 1992), transmission of ZYMV was higher than WMV-2 from single and mixed infections by both *M. persicae* and *A. gossypii*. Interestingly, transmission rates of ZYMV by *M. persicae* from single and mixed infections declined in 4 weeks, while transmission of WMV-2 increased in the same period. Transmission rates of *A. gossypii* remained stable for both viruses.

In a similar study, Webb (1992) found that transmission *of* WMV-2 by *M. persicae* was related to virus titer in the plant. In general, only leaves showing symptoms were reliable sources of inoculum in multiple-vector-transfer tests. Transmission of poorly aphid transmissible ZYMV isolates and WMV-2 in mixed

infections has been studied by Lecoq et al (1991a) and is mentioned elsewhere in this chapter.

M. persicae and *A. gossypii* are also believed to be responsible for transmission of WMV-2 and cucumber mosaic virus in spring cantaloupes in southwestern and central Arizona (Nelson and Tuttle, 1969). In Arkansas, *A. craccivora* and *A. gossypii* prevailed over the other three aphid vectors found in fall squash infected with WMV-2 (Hander et al., 1993). In Japan, Yamamoto et al. (1986), attributed the spread of WMV-2 among cucurbit fields to at least 14 of 23 known aphid vectors occurring in that country. *Aphis gossypii*, however, was considered to play a major role in primary and secondary viral dispersion. In northern India, the same aphid was reported to be the most important vector, surpassing *M. persicae* in its efficiency of transmission of PRSV-W to cucurbit crops (Tewari, 1976).

Webb and Kok-Yokomi (1993) first reported *Uroleucon pseudambrosiae* as a vector of WMV-2 and PRSV-W, suggesting that this aphid would play an important role in spring epidemics of these viruses in watermelon in Florida. This species was able to transmit WMV-2 (isolate G2301) in arena (23% ± 12% SD, N=15) and controlled tests (53 out of 450 plants tested). PRSV-W was also successfully transmitted in arena tests (41% ± 22 SD, N=10). An aphid-transmissible (*M. persicae*) Florida isolate of ZYMV was not transmitted by *U. pseudambrosiae*, either in arena tests or in controlled access tests.

In a further study (Webb et al., 1994), in which the effect of trap color on species composition of alate aphids caught was assessed, it was suggested that the trapping method would affect one's conclusions regarding the importance of each species. Thus, the role of *U. pseudambrosiae* in epidemics of WMV-2 and/or PRSV-W may have been overlooked in previous studies (Adlerz, 1987;1974a), and the importance of *A. spiraecola* may have been overemphasized due to of its strong attraction to yellow. Webb and coworkers (1994) confirmed previous findings that green tile water pan traps

could be more reliable for estimating species composition of alate aphids landing in a watermelon crop because the spectral reflectance of the green tiles more closely matched that of green leaves than that of the yellow traps (Irwin and Schultz, 1981). Boiteau (1990), however, suggested that although green tile traps improve sampling reliability compared with yellow water pan traps, some sampling bias may occur nevertheless. In contrast, potato leaf water pan traps were found to be the best method to estimate aphid landing rates in potato. However, because it was difficult and time-consuming to use this trap, Boiteau (1990) suggested the green tile water pan trap for epidemiological studies.

Transmission Test: Estimation of Vector Propensity and Vector Efficiency

Modeling epidemics of plant virus diseases transmitted by aphids relies, to a great extent, on the composition and abundance of potential vectors and viral transmission rates of individual species. Estimation of transmission rates is essential for simulation models (Irwin and Ruesink, 1986; Ruesink and Irwin, 1986; Plumb et al., 1986, Watson and Healy, 1953) and for comparative studies involving virus sources, test plants, vectors, and virus strains or isolates (Yokomi et al., 1994; Webb and Kok-Yokomi, 1993; Labonne et al., 1992; Fereres et al., 1992; Swallow, 1985).

The Transmission Rate and Related Terms

The transmission rate of a nonpersistent virus can be defined as the "percentage of aphids which are able to contaminate a sensitive plant after probing an infected plant" (Labonne et al., 1992, p. 269). A more descriptive definition of transmission rate (Swallow, 1985; Sylvester, 1954) is the probability (p) of virus transmission by a single vector under a specified set of conditions.

Berger and Ferriss (1989, p. 42) use the term *transmission efficiency* instead of *transmission rate*, and it is defined as "proportion of a vector population that transmits virus. Represents the ability of a given population or sample of vectors to transmit a particular virus; usually expressed as percentage".

Depending on how the transmission rate is estimated, several terms have been suggested to define the biological event that transmission rate is assessing.

If the transmission rate is estimated with complete control over the transmission process, i.e., timed acquisition access period and/or timed inoculation access period, then the term *vector efficiency* has been suggested (Sylvester, 1954).

If the transmission rate is estimated without control over the acquisition access period, the behavior of the vector is taken into account, and the term *vector propensity* can be applied (Irwin and Ruesink, 1986). Both *vector efficiency* and *vector propensity* may imply experimentation. Their differences depend on the extent of experimental manipulation. Much of the literature is comprised of aphid transmission studies that either explicitly or implicitly estimate transmission rate through these two biological events (Yokomi et al., 1994; Webb and Kok-Yokomi, 1993; Fereres et al., 1992; Labonne, 1992; Risser et al.,1981; Paguio and Kuhn, 1976; Watson and Plumb, 1972).

Other terms related to transmission rate have been suggested. *Vector competence,* for example, can be thought of as the genetically inherent determination of transmission rate without enviromental effect (Gold, 1979).

Another term is *infectivity index* (Plumb et al., 1986). In essence, this term describes the product of vector propensity of a particular aphid and its population density (counts of individuals). Because estimation of the transmission rate is not fixed through the growing season (Plumb et al., 1986), this term is not analogous to *vector intensity*, a similar term proposed by Irwin and Ruesink (1986).

Vector potency was suggested by Sylvester (1954) to measure the level of virus charge acquired under various feeding conditions, including serial transmission.

Transmission of Potyviruses

Potyviruses (Potyviridae:*Potyvirus* genus) are transmitted nonpersistently by aphids (Shukla et al., 1994; Edwardson, 1992; Berger, 1992; Berger and Ferriss, 1989). The characteristics of transmission of potyviruses are listed in Table 2.5. Groups of viruses taxonomically related to the potyvirus genus have other mechanisms of transmission: *Bymovirus* (fungus) and *Rymovirus* (mites) with five viruses each, and *Ipomovirus* (whiteflies), a possible taxon, with one virus. In addition to these groups, there are 17 viruses whose vectors are unknown (Shukla et al., 1994; Edwardson, 1992; Zettler, 1992; Edwardson and Christie, 1991).

Aphid transmission of potyviruses involves the interaction of charged virus particles (Govier and Kassanis, 1974) and/or coat protein (Berger, 1992) and helper component protein with the aphid stylet and the surfaces of the alimentary canal anterior to the esophageal valve (foregut) (Berger and Pirone, 1986). This event may take place intracellularly in the epidermal or mesophyll layer in the host plant tissue, and less frequently, intercellularly (Blua and Perring, 1992; Powell, 1991; Lopez-Abella et al., 1988). In the latter case, possible acquisition and inoculation of the virus could be via broken plasmodesmata (Nault and Gyrisco, 1966).

The lowest transmission threshold reported, i.e., the combined minimum acquisition and inoculation access period under controlled conditions (Sylvester, 1954), is approximately 11 seconds (*Nicotiana tabacum* - potato virus Y - *M. persicae* system) (Powell, 1991) (Table 2.5). Berger and Ferriss (1989) reported 10 seconds for potyviruses in general. These thresholds suggest that, in nature, perhaps a single probe in the source and in the healthy plant are enough to transmit a virus. Experimentally, natural probes can last between 15 to 60 seconds as observed using a hand lens or microscope (Summers et al., 1990; Schultz, 1983; Paguio and Kuhn,

1976), or 7.6 (acquisition) and 3.6 (inoculation) seconds, as determined with an electronic feeding monitor (Powell, 1991).

The transmission of potyviruses seems to be unique in that a helper component is required for successful transmission (Huet et al., 1994; Granier et al., 1993; Gal-On et al., 1992; Lecoq et al., 1991a; Lecoq, 1986; see section WMV-2, ZYMV and PRSV-W Aphid Vectors), and that puncturing epidermal and mesophyll cells occurs without damage to the plasmalemma (Powell, 1991; Lopez-Abella et al.,1988). The latter attribute may be typical of all nonpersistently transmitted viruses (Lopez-Abella et al., 1988).

Table 2.5. Experimental conditions under which potyviruses have been transmitted by various aphid species (Homoptera: Aphididae).

Stage of transmission[1]	Characteristics[2]	References
preacquisition starvation period	0 - 24 hrs	Fereres et al.,1992; Jensen, 1949
acquisition access period	single probe (7.6 s)-24 hrs	Webb & Kok-Yokomi,1993; Powell, 1991; Summers et al.,1990; Shultz, et al., 1983; Paguio and Kuhn, 1976; Jensen, 1949
postacquisition starvation period[3]	0 - 30 hrs	Fereres et al.,1992; Jensen 1949; Sylvester, 1954
latent period	0	Berger and Ferriss, 1989
inoculation access period	3.6 s - 48 hrs	Powell, 1991; Fereres et al.,1992; Labonne et al.,1992; Paguio and Kuhn, 1976; Jensen, 1949; Sylvester, 1954; Watson, 1936
transstadial passage	none	Berger and Ferriss, 1989
transovarial passage	none	Berger and Ferriss, 1989
serial transmission	in some systems	Tewari, 1976; Paguio and Kuhn, 1976; Jensen, 1949; Watson, 1936

[1] Definition of most of these terms can be found in Berger and Ferriss (1989).
[2] Values represent the range at which tests have been conducted and giving positive results.
[3] Starvation period at which an aphid remains viruliferous after acquiring the virus and infects a healthy plant.

The general process of nonpersistent transmission is determined by many factors. The effects of several biological factors have long been recognized as playing an important role in the transmission rate estimates in several nonpersistent plant-virus-vector systems (Watson, 1936; Sylvester, 1954). With potyviruses, it has been shown that transmission rates can be influenced by genetic characters, physiological phase, and behavior of the aphid (Blua and Perring, 1992), host genotype (Risser et al., 1981; Gray et al., 1986; Romanow et al., 1986), environment (Fereres et al., 1992, Labonne et al., 1992; Watson, 1936), and concentration, purity of infection, and strain of the virus (Huet et al., 1994; Granier et al., 1993; Webb, 1992; Castle, 1992; Lecoq and Purcifull, 1992; Lecoq et al., 1991a; Lecoq, 1986; Risser et al., 1981; Paguio and Kuhn, 1976). Therefore, evaluation and standardization of some of these components has to be done for the purposes of comparative studies, regardless of the method to be used to estimate the transmission rate.

Four general methods have been used to determine transmission rates: laboratory live assays, field live assays, serological methods, and plant traps. Advantages and disadvantages of these approaches are fully described by Raccah and Irwin (1988), Irwin and Ruesink (1986), and Raccah (1983). Because of their broad use in comparative studies, detection of potential vectors, and possible application in quantitative epidemiology, two live laboratory assays will be addressed in this section: controlled access and the arena test.

Controlled Access Test

Controlled access tests, either the single-vector-transfer or multiple-vector-transfer method, have been extensively used in transmission experiments with aphids (Yokomi et al., 1994; Webb and Kok-Yokomi, 1993; Labonne et al., 1992; Fereres et al., 1992; Lambert, et al., 1990; Gibbs and Gower, 1960; Sylvester, 1954; Watson,

1936). Access periods for either acquisition or inoculation, or both, are usually controlled in these types of tests. The term access period (for acquisition), as defined by Sylvester (1954), is the "period of time during which a vector has access to a virus source, but during which feeding for the entire allotted time may not occur." Similarly, the term inoculation access period can be thought of as the period of time during which a vector has access to a healthy test plant, but during which feeding may not occur for the entire time. The application of these terms varies in the literature. In some papers these terms were used as defined by Sylvester (Webb and Kok-Yokomi, 1993; Fereres et al., 1992; Labonne et al., 1992), whereas in others actual probing in the source tissue or feeding in the test plant was taken into account (Summers et al., 1990; Schultz et al., 1983; Paguio and Kuhn, 1976; Swenson, 1964; Swenson, 1957). In some cases an overnight to 24-h acquisition access period was provided (Koike, 1979).

It has been suggested that trials have to be performed synchronously over a short period of time to allow comparison of a series of experiments (Watson, 1936). A recent study, however, indicates that time and synchronicity need not be a restrictive factor (Labonne et al., 1992). Labonne and coworkers (1992) determined in the system *Cucurbita pepo*- PRSV-W -*Aphis gossypii* that the transmission rate was a stable property independent of the trial date as long as fasting, acquisition access period, stage of the aphids, and temperature and light conditions of the aphid colony were standardized. Temperature during the acquisition and inoculation access periods, the plant used to rear the aphid colony, and the age of the adult aptera over a 1-7 day period did not have a significant effect on the transmission rate.

Standardization of transmission tests with nonpersistent viruses is dependent upon the system studied and optimum conditions have been established by pioneer researchers (Watson, 1936; Sylvester, 1954). Tewari (1976) determined optimum transmission of PRSV-W by *A. gossypii* to *C. pepo* with 4-h preacquisition starvation-period time, 2-min acquisition access time, no postacquisition starvation time, and a 24-

h inoculation access period (in the range of 2-48 hours). Fereres et al. (1992) found that transmission of ZYMV by *A. gossypii* to *C. pepo* was optimum when tests were conducted with a 1-h preacquisition starvation time, 5-min acquisition access time, and moderate temperature (21°C) / high relative humidity (80%), whereas transmission of ZYMV by *Myzus persicae* was not influenced by any of these factors. Webb and Kok-Yokomi (1993) indicated that transmission of WMV-2 to *Citrullus lanatus* by *Uroleucon pseudambrosiae* apparently increased with longer inoculation access period, but was not affected by starvation and acquisition access time.

Controlled Access Test: Single-Vector-Transfer Method

Controlled access tests involving potyvirus transmission with the single-vector-transfer method have been extensively conducted (Summers et al., 1990; Paguio and Kuhn, 1976; Tewari, 1976; Swenson et al., 1964). Acquisition access by several aphids to a single leaf source has usually been used (Webb and Kok-Yokomi, 1993; Labonne et al., 1992; Fereres et al., 1992; Schultz et al., 1983). Because the transmission rate can be determined directly with the fraction of test plants that develop symptoms (Swallow, 1985; Gibbs and Gower, 1960), single-vector-transfers are often used to determine transmission rates in comparative studies under different experimental conditions. However, in some conditions, consideration of cost and convenience makes the single-vector-transfer method impractical (Swallow, 1985; Gibbs and Gower, 1960).

Controlled Access Test: Multiple-Vector-Transfer Method

Controlled access tests with groups of aphids have also frequently been used with potyviruses. Groups of 2-20 aphids [200 used by Jensen (1949)], have been used for conditions under which the probability of transmission is low (Sylvester, 1954), and to identify aphid species or stage of the vector capable of transmitting a particular potyvirus (Lambert et al., 1990; Risser et al., 1981; Koike, 1979; Tewari, 1976;

Swenson, 1957; Coudriet, 1962; Jensen, 1949). This type of test can be referred to as *multiple-vector-transfer* method after Gibbs and Gower (1960) and Swallow (1985). In some of these studies, the transmission rates were implicitly estimated by using the same criterion that has been used in controlled tests with the single-vector-transfer method. Although this approach has been successfully used in comparative studies, provided that the number of aphids is standardized (Risser, et al., 1981; Koike, 1979; Tewari,1976; Watson, 1936), this is not an appropriate approach for modeling of virus epidemics where the vector intensity, *sensu* Irwin and Ruesink (1986), is a requisite.

Swallow (1985) considered that treatment differences should be judged using transmission rate values, and not by comparing observed fractions of positive tests, even when the same number of vectors were used. This is because the number of infected plants is a direct function of the number of aphids used as well as the experimental conditions.

Watson (1936) showed that the numbers of plants infected with henbane mosaic virus by *M. persicae* increased with the number of aphids used per test plant. Similar results were reported by Yokomi et al. (1994) for both *Toxoptera citricida* and *A. gossypii* in transmission tests with the closterovirus citrus tristeza virus, and Mexican lime (*Citrus aurantifolia*).

Tewari (1976), however, found no increase in the number of plants infected after five individuals of *A. gossypii* were used to transmit PRSV-W in *C. pepo*, and Kirkpatrick and Ross (1952) stated that viruliferous *M. persicae* transmit potato leafroll virus more frequently when caged alone than when caged together with other non-viruliferous aphids.

Kirkpatrick and Ross (1952) suggested that multiple aphids induced plant resistance to systemic infection and/or interference in feeding. A later study, conducted by Bindra and Sylvester (1961) proved that, in fact, potato leafroll virus infection increased with the number of aphids tested.

Watson (1936) estimated the expected number of plants infected with the equation:

$$1 - q^x$$

where q is the probability of non-infection and x is the number of aphids used in the test. The q-values were estimated by maximum likelihood solution of the binomial theorem. Watson (1936) concluded that each aphid transmission may be "assumed to be essentially local (on the tissue) and independent." Therefore, transmission is not believed to be additive to individual dosages or virus charge in a single vector (Sylvester, 1954).

Gibbs and Gower (1960), following an approach similar to that used by Watson (1936), determined the transmission rate with the following equation:

$$p^* = 1 - (1 - R/N)^{1/i}$$

where p^* is an estimator of the transmission rate per aphid within the group, R is the number of test plants infected, N is the total number of tests plant used, and i is the number of aphids in the group. Gibbs and Gower (1960) extended the application of the equation for determining the transmission rate in mechanical inoculation of groups of plants. This equation form has been used in several comparative studies (Yokomi et al., 1994; Hunt et al., 1988). No attempt has been made to use estimates of transmission rates from group testing for the purpose of modeling temporal disease spread.

A drawback of the multiple-vector-transfer method is that when i is too high, the standard error and bias are also high making p^* useless (Gibbs and Gower, 1960). Swallow (1985) illustrated a method to overcome this problem and provided a table to select the best combination of number of test plants and number of aphids to minimize

the standard error and bias. Swallow (1985) concluded that in most cases it is easy to select a multiple-vector-transfer design; though not optimal, this design is both safe and an improvement over a single-vector-transfer experiment.

Regardless of the controlled access test performed, i.e., single-vector-transfer or multiple-vector-transfer, two characteristics are measured under a determinate set of conditions: the biological properties of the virus, related to its dispersal potential, and the biological characteristics of the vector (Labonne et al., 1992). Because the behavioral attributes of the vector are not fully taken into account in controlled access tests, the transmission rate estimated in this way can be thought of as a measurement of *vector efficiency* (Irwin and Ruesink, 1986).

Arena Test

An alternative assay that incorporates the behavioral freedom of movement and probing duration and frequency in the estimation of the transmission rate is the arena test (Irwin and Ruesink, 1986). At least three drawbacks have been recognized in the arena tests: aphid movement is restricted in a confined space; clones (aphids descended parthenogenetically from a single aphid, thus genetically identical) instead of wild specimens are usually used; and early plant phenological stages (i.e. seedlings) are commonly tested (Irwin and Ruesink, 1986). It may be argued that the latter two drawbacks are also present in controlled tests and, to some extent, in live field assays (Webb and Kok-Yokomi, 1993; Labonne, 1992; Castle et al., 1992; Adlerz, 1987). Nevertheless, Irwin and Ruesink (1986) advocate the use of this assay because it allows estimation of vector propensity, a vector attribute of natural transmissibility. Thus, its utilization in modeling virus epidemics may have great potential.

The arena test has not been extensively used, however (Webb and Kok-Yokomi, 1993; Summer et al., 1990). Although it has been shown that the transmission

threshold (*sensu* Sylvester, 1954) for nonpersistent potyviruses is in the range of 10-11.2 seconds (Powell, 1991; Berger and Ferriss, 1989), the flexibility in the process of acquisition-movement-inoculation seems to be essential to the study of some host-virus-vector systems. Summers et al. (1990) found that *Acyrthosiphon kondoi* Shinji and *Diuraphis noxia* (Mordvilko) transmitted beet mosaic virus (a potyvirus infecting *Beta vulgaris*) in arena tests, but not in controlled acquisition and inoculation access period tests.

Success of comparative studies with the arena test has been obtained by using the proportion of infected plants (Webb and Kok-Yokomi, 1993; Summers et al., 1990). However, by analogy with the problem of using this approach in multiple-vector-transfers, it seems that the use of transmission rates could be the safer method for comparison of experimental conditions. The same evidence discussed in the context of multiple-vector-transfer method should be valid here. That is, the number of infected plants is a direct function of the number of aphids used as well as the experimental conditions (Swallow, 1985). There is, however, one additional piece of evidence against using the fraction of infected plants in comparison of treatments in arena tests. Because a group of aphids are released on a virus source and allowed free acquisition and inoculation to a set of surrounding plants in an enclosed environment (Irwin and Ruesink, 1986), the possibility for serial transmission exists.

Serial transmission has been demonstrated by using special arrays in single- and multiple-vector-transfer tests and seems to be a function of the plant-virus-vector system.

Serial transmission of viruses has been reported with *M. persicae* transmitting henbane mosaic to *Nicotiana* sp, brassica nigra virus (= turnip mosaic virus)(Smith, 1972) to *Brassica juncea*, and PRSV-P to *Carica papaya* (Sylvester, 1954; Jensen, 1949; Watson, 1936); with *A. gossypii* transmitting PRSV-W to *C.pepo* (Tewari, 1976);

and with *M. persicae* and *A. craccivora* transmitting peanut mottle to *Arachis hypogaea* (Paguio and Kuhn, 1976).

Although the transmission rate is intuitively attractive for comparative studies involving arena tests, there are no any statistical models proposed in the literature to estimate this parameter. It has been stated that arena tests could be used to estimate vector propensity (Irwin and Ruesink, 1986); however, this is only true to the extent that transmission rate could be estimated for each individual. This would allow possible application of the transmission rate to simulation of virus epidemics in addition to comparative studies. To date no attempts have been made either to derive a statistical method to estimate the transmission rate or to apply this parameter in epidemiological modeling. Thus, this is an open field of research in plant virus epidemiology.

Epidemics of WMV-2, PRSV-W, and ZYMV: Temporal Studies

Temporal analysis of epidemics, i.e., the study of changes in disease incidence or severity over time in a population of plants, is important to determine the speed of an epidemic under certain conditions (Campbell and Madden, 1990; Raccah, 1986). This approach is fundamental in epidemiological studies when the goal is to discriminate, among a series of factors, those more important in driving the development of an epidemic. Thus, the integration of temporal studies with other approaches aids in selecting a control measure and in applying it at the proper time.

Potyviruses cause severe epidemics in cucurbits and other crops throughout the world (Shukla et al., 1994; Raccah, 1986). Although this group of viruses plagues many plant species (Edwardson and Christie, 1991), severe epidemics have been reported in the Cucurbitaceae, Solanaceae, and Fabaceae families (Webb and Linda, 1993; Nelson and Campbell, 1993; Hander et al., 1993; Castle, 1992; Lecoq et al.,

1991b; Madden et al., 1987b; 1987c; Gray et al., 1986; Adlerz 1978a; Ruesink and Irwin, 1986; Sigvald, 1986; Raccah et al., 1988; Nelson and Tuttle, 1969).

Potyviruses are well adapted to temperate climates (Lecoq and Purcifull, 1992). However, epidemics incited by some of these viruses have been reported in tropical and subtropical crops, such as sesame (*Sesamum indicum*), papaya (*Carica papaya*), pepper (*Capsicum annuum*), and sugarcane (*Saccharum officinarum*) (Sreenivasulu et al., 1994; Mora-Aguilera et al., 1995, 1993b, 1992; Marco et al., 1993; Grisham, 1993; Basky and Raccah, 1990).

Seasonal Occurrence and High Incidence: Two Characteristics of Epidemics

The temporal spread of WMV-2, PRSV-W, and ZYMV in cucurbit crops has been studied in the United States in Florida (Adlerz, 1974a; 1978a; Webb and Linda, 1993), North Carolina (Gray et al., 1986), Arkansas (Hander et al., 1993), Arizona (Nelson and Tuttle, 1969), California (Castle, 1992; Perring et al., 1989); and in other countries such as Nepal (Dahal, 1992), France (Lecoq et al., 1991b), and Taiwan (Wang et al., 1991).

Occurrence of epidemics, but not specific epidemiological studies, have been reported from Connecticut (Provvidenti et al.,1984), Hawaii (Ullman et al., 1991), New Jersey (Davis and Mizuki, 1987), and México (Orosco-Santos et al., 1994).

In the first epidemiological study of WMV-2 infecting cucurbits in North Central Florida (Adlerz, 1978a), it was shown in four consecutive seasons that between 57 and 99% of plants became infected with WMV-2 when epidemics began early. When epidemics began late (about 6 wk before harvest) incidence did not reach 5%. No additional epidemiological parameters were reported in this study.

A recent study of the effect of oil and an insecticide on epidemics of WMV-2, ZYMV, and PRSV-W in watermelon documented epidemics with typical sigmoidal

logistic curves (Webb and Linda, 1993). Although the average apparent infection rates of these epidemics were not estimated (location-shifted parameters were estimated instead), a good fit to the logistic model was reported for all epidemics.

A graphical inspection of the three seasons (fall 1988, spring 1989 and 1990) clearly shows that the natural epidemic (plot without oil or insecticide) reached almost 100% incidence of disease in 30-40 days (Webb and Linda, 1993). These estimates were higher than those reported earlier by Adlerz for the same region during spring epidemics (Adlerz, 1978a). Although epidemics started later in the spring season, no differences in disease progress were found between spring and fall plantings but the composition of viruses infecting plants differed (Table 2.1).

Seasonal differences in epidemics induced by WMV-2 in three genotypes of *Cucumis melo* in North Carolina, were reported by Gray et al. (1986). High average apparent infection rates (0.027-0.049 units $days^{-1}$), high final disease incidences (92%-100%), epidemic duration time of about 35 days, and no differences among epidemic progress curves among genotypes were detected in summer plantings (6 August). In contrast, low infection rates (.0033-0.0358 units day^{-1}), low final disease incidences (11% - 65%), and differences in epidemic progress curves among genotypes were found in the spring (27 April). Seasonal differences were attributed to an increase in the number of alighting aphids and sources of WMV-2 in the surrounding area. Neither identification of aphid species nor determination of WMV-2 sources were reported in the study (Gray et al., 1986).

Seasonal and regional effects on epidemic development were also reported by Hander et al. (1993). Final disease incidences of 23.2% (WMV-2) and 92.8% (PRSV-W) were found in late summer squash plantings in south central (August 19) and western Arkansas (September 2), respectively. Greater spread of disease in late summer was attributed more to the proximity of sources of inoculum (fields infected) than to abundance of aphid vectors (Hander et al., 1993). No plants infected with

WMV-2, ZYMV, or PRSV-W were detected in spring plantings (April 11), even though counts of aphid vectors caught were higher than those observed in the autumn.

Sources of Inoculum: an Important Role in Epidemics

In south central and western Arkansas (Hander et al., 1993) and the western United States, potyvirus epidemics have also been attributed to the distribution and prevalence of virus source plants more than to the abundance of aphid vectors.

Because counts of total aphids were not correlated (empirically estimated) with mosaic virus incidence, Nelson and Tuttle (1969) suggested that spring epidemics of WMV-2 and cucumber mosaic cucumovirus (CMV) in *Cucumis melo* were dependent upon the abundance of alternative hosts restricted to irrigated land and gardens in southwestern Arizona. Three out of six possible alternative hosts, including cultivated annuals, weeds, and perennial ornamental plants, were suggested as important sources of WMV-2. Five epidemics from different seasons had final disease incidences in the range of 75 to 100%, and the duration of epidemics ranged from 35 to 60 days. All five epidemics exhibited typical sigmoidal curves. Two other epidemics reached 20 and 40% incidence.

Adlerz (1974a) also reported no correlation of initial spread of WMV-2 or PRSV-W with first flights of any of the vector species trapped. These studies were not statistically verified, however. Abundance of weed hosts, on the other hand, was apparently associated with the initial spread of infected plants. Infected weeds, upwind within 15-38 meters from fields, were reported to be the source for primary infections of PRSV-W in South Florida (Adlerz, 1974b; 1972a; 1972b).

In southern California, where ZYMV and WMV-2 are endemic in spring muskmelon fields (Perring et al. 1989), the same lack of "simple relationship" between virus incidence and numbers of aphids has been reported by Castle (1992).

Regardless of the abundance of aphids, proximity of inoculum sources to commercial fields was identified as the main cause of virus spread. Although WMV-2 spread faster than ZYMV in three out of five fields, typical logistic sigmoidal curves and final disease incidences of 100% for WMV-2, or WMV-2 and ZYMV were observed from independently monitored epidemics for each virus. Total epidemic time ranged from 10 to 46 days. Prevalence of WMV-2 over ZYMV was attributed to abundance of alternative hosts for the former virus.

In France, opposite results were reported by Lecoq (1991b) in a two year study. Although both viruses reached 100 % incidence in 42-49 days, ZYMV spread faster than WMV-2.

The general characteristics of potyvirus epidemics in cucurbits of southeastern and western United States, i.e., typical sigmoidal logistic curves, seasonal variation, up to 100% final disease incidence, epidemic duration times ranging from 10 to 60 days, and dependence upon abundance and closeness of sources of inoculum, appear to be applicable to other countries, including Nepal (Dahal, 1992), France (Lecoq et al., 1991b), and Taiwan (Wang et al., 1991).

It should be noted that the lack of correlation between disease incidence and abundance of aphids reported by some researchers was based upon empirical observations, rather than on statistically verified studies.

Papers demonstrating statistical or biological correlations between disease incidence and vector density will be discussed later in this chapter in the forecasting section.

Analysis of Spatial Patterns: Basic Theory and Approaches

Populations change in two dimensions, time and space (Campbell and Madden, 1990; Madden and Campbell, 1986; Pielou, 1969). Change in space is an important

attribute that can be used to generate hypotheses about the structure of populations (Ludwing and Reynolds, 1988). Several factors are involved in the spatial pattern of organisms (Hutchinson,1954): (1) vectorial factors resulting from the action of external environmental forces (e.g., wind, water currents, and light intensity); (2) reproductive factors attributable to the reproductive mode of the organism; (3) social factors; (4) coactive factors resulting from intraspecific interactions (e.g., competition); and (5) stochastic factors resulting from random variation in any of the preceding factors.

The characterization of the spatial pattern of a population and its possible causes may lead to practical applications. In plant virus epidemiology, spatial patterns are studied to assist in determining the source of inoculum, identify factors involved in disease spread, assess disease intensity properly, validate assumptions for multiple regression models in forecasting, and aid in defining control measures adequately (Nelson and Campbell, 1993; Mora-Aguilera, 1992; Campbell and Madden, 1990; Chellemi et al., 1991; Lecoustre et al., 1989; Madden, 1989; Madden et al., 1987b; Madden and Campbell, 1986).

Two general approaches can be used to study spatial attributes of populations (Campbell and Madden, 1990; Madden and Campbell, 1986).

In one approach, short- or long-range dispersal of an organism from a localized focus or site in space and the resulting gradient are examined. Reference to this approach is usually found in the literature as *analysis of spatial dispersion.*

In the second approach, the spatial pattern of an organism is described. This approach is often referred as *analysis of spatial patterns.* Either approach may be uni- or bi-dimensional in space and can be studied at a static time or at several times to give a spatio-temporal characterization.

Several specific techniques can be used in each approach to characterize the spatial attribute of a population. These techniques are given in Table 2.6.

Table 2.6. Techniques used to characterize the spatial attributes of a population in plant disease epidemiology and ecology.

Techniques	References
Unidimensional Spatial Patterns	
Doublet analysis	Vanderplank (1946)
Runs analysis	Madden et al. (1982)
Bidimensional Spatial Patterns	
Gray's two-dimensional distance class analysis	Nelson et al. (1992)
Dp (Pielow index), DHS, DCE	Pielou (1969)
nearest-neighbor analysis	Clark and Evans (1954)
geostatistics	Chellemi et al. (1988)
Aggregation Indexes	
variance-to-mean ratio, Lloyd's index of patchiness	Pielou (1969), Lloyd (1967)
Morisita's index, Iwao's b, Taylor's power law	Morisita (1964), Iwao (1968), Taylor (1961)
Greig-Smith's technique	Greig-Smith (1952,1983)
autocorrelation analysis	Reynolds and Madden(1988), Gottwald et al (1992)
Discrete distributions	
Poisson and Thomas double Poisson	Campbell and Noe (1985)
negative and positive binomial	Pielou (1969); Greigh-Smith (1983)
Newman type A	Pielou (1969)
Beta-binomial	Hughes and Madden (1993)
Ecological Maps	
L-mosaic, S-mosaic	Pielou (1969)
Spatial Dispersal	
Empirical models	
exponential model	Frampton et al (1942), Kiyosawa and Shiyomi (1972)
power low	Gregory (1968), Gregory and Read (1949)
Wadley and Wolfenbarger's model	Wadley and Wolfenbarger (1944)
Continuous Distributions	
diffusion models	Rudd and Gandour (1985), Shigesada (1980), Okubo (1980,1986,); Okubo and Chiang (1974); Aikman and Hewitt (1972)
stationary wave models	Fisher (1937)

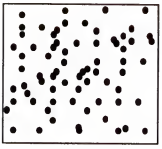
The ecological theory behind the pattern or dispersion of a population through a definite space, takes into account three scenarios (Pielou, 1969):

- Cases in which the organisms are confined to discrete sites or "units".
- Cases in which the organisms have a continuous space that can be occupied.
- Cases in which the organisms have a continuous space that can be occupied, but there is not a clear delimitation of individuals that can be counted.

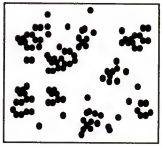
These scenarios may determine the technique of analysis to be used in spatial studies. For example, aggregation indexes, Greig-Smith's method, and ecological mapping can be used for cases 1, 2, and 3, respectively. Spatial models in virus epidemiology have been based on the assumption that the system is open, so that individuals move into or out of the spatial region, and colonization may or may not occur (Nelson and Campbell, 1993; Mora-Aguilera et al., 1992; Chellemi et al., 1991; Lecoustre et al., 1989; Madden, 1989; Madden et al., 1987b; Madden and Campbell, 1986). This assumption is associated with case 2 described by Pielou (1969).

The analysis of spatial patterns, either uni- or bi-dimensional, leads to identifying specific patterns through the different analytical methods. Three basic types of spatial patterns are recognized in a population of organisms: random, aggregated or clumped, and uniform (Campbell and Madden, 1990; Madden, 1989; Ludwig and Reynolds, 1988; Pielou, 1969) (Figure 2.1). Random patterns imply environmental homogeneity and /or nonselective behavioral patterns. Nonrandom patterns (clumped and uniform) imply that some constraints on the population exist. Clumping suggests that individuals are aggregated in more favorable parts of the habitat due to gregarious behavior, environmental heterogeneity, reproductive mode, or other factors (Patterson, 1993; Irwin and Kampmeier, 1989). Uniform dispersion results from negative interactions among individuals, such as competition for food or space (Ludwig and Reynolds, 1988). Spatial patterns are dependent upon the choice of scale. A particular pattern may be

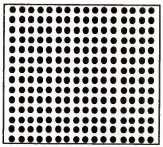
detected at one scale but not at another. Some techniques take this aspect into account by exploring patterns at various scales in the community (Ludwig and Reynolds, 1988).



(a) Random



(b) Clumped



(c) Uniform

Figure 1.1 Three types of spatial patterns: (a) random, where all individuals are located independently of each other; (b) clumped, where individuals tent to locate together in clusters; and (c) uniform, where individuals are regularly spaced.

Analysis of Spatial Patterns: Nonpersistent Viruses

Interpretations of spatial patterns of virus diseases have been given by Madden et al. (1987a; 1987b), Madden (1989), and Thresh, (1974). An initial random pattern and a final aggregated pattern during the progress of an epidemic is characteristic of virus diseases that are transmitted nonpersistently by aphids (Madden et al.,1987a; 1987b; Thresh, 1974). A random pattern may be due to primary infection by alate aphids from external virus sources (Mora-Aguilera et al., 1992). An aggregated pattern may be caused by spread of the virus by colonizing aphids within the field (secondary infection) (Madden et al., 1987b). The absence of external sources of inoculum may be

another characteristic of random patterns (Converse et al.,1979; Madden et al., 1987a). Regular patterns apparently have not been observed in viruses transmitted nonpersistently by aphids.

Several attempts have been made to biologically explain the dispersion pattern of potential aphid vectors which could, in turn, help to elucidate distribution patterns of diseased plants. Patterson (1993) suggested, based on olfactometer tests and field trapping studies, that the aggregation pattern of gynoparae of *Rhopalosiphum padi* was due to an aggregation pheromone released by primary colonizers. Primary colonizers appeared to settle on the basis of food quality.

Other types of stimuli may play important roles in aggregation behavior and settling such as tactile and visual stimuli (Irwin and Kampmeier, 1989). Colors may induce settling and landing in some cases (Taylor, 1986). Webb et al. (1994), demonstrated differences in species composition and abundance in comparative studies with yellow and green water pan traps in watermelon fields. Yellow traps, in general, attracted more aphids, *A. spiraecola* being the most abundant. This aphid was 45 to 163 times more attracted to yellow than to green.

Blua and Perring (1992), who monitored feeding electronically, demonstrated that alates of *A. gossypii* rejected *Cucurbita pepo* plants that were infected for 4 weeks with ZYMV, a virus that induces strong yellowing symptoms soon after infection (Adlerz et al., 1983). Healthy plants or plants infected for only two weeks were readily colonized. The feeding process was also influenced by the quality of the host. Aphids expended more time in salivary sheath formation and less time in phloem feeding in plants infected for 4 weeks.

Other aphids, however, colonize diseased plants more readily than healthy ones. Baker (1960) showed that *Myzus persicae, M. ascolonicus, A. fabae,* and *Aulacorthum solani* preferred yellows-virus-infected leaves of sugar beet. These aphids reproduced rapidly and lived longer on tissue with the most severe symptoms.

Colonization appears to be an explanation of spatially aggregated patterns observed for some aphid species (Madden et al., 1987b; Gray et al., 1986); conversely, transient aphids appear to induce random patterns as indicated by variance-to-mean ratio and Lloyds's index of patchiness (Mora-Aguilera et al., 1992). The mechanisms underlying the spatial patterns are not fully understood. Examination and analysis of spatial patterns is not straightforward, and often interpretation of results has to be done by integration of several techniques (Gottwald 1994, personal communication).

The variance-to-mean ratio, Lloyd's index of patchiness, and the Greig-Smith technique estimated from contiguous quadrats have been used to characterize the epidemics caused by tobacco etch virus and tobacco vein mottle virus in tobacco (Madden et al., 1987b) and PRSV-P in papaya (Mora-Aguilera et al., 1992). A disadvantage of these approaches is that quadrat size can influence the interpretation of results and thus may lead to false conclusions (Campbell and Madden, 1990). Mora-Aguilera et al. (1992) showed that, even though the method of Greig-Smith was used to determine the optimum quadrat size, results varied according to the direction of quadratization.

The two-dimensional distance class analysis approach was first employed by Gray et al. (1986) to characterize WMV-2 epidemics. This method quantifies two-dimensional patterns of infected plants within a given field based upon distances between those plants. Important features of this approach include identification of edge effect, cluster size, and localization (Nelson et al., 1992). These epidemiological features are not provided by other approaches, including the Morisita index, Lloyd's idex of patchiness, ordinary run analysis, and first-order autocorrelation.

Although the two-dimensional spatial analysis does not require definition of quadrats and thus is not affected by quadrat size, the result can be affected by low (< 10%) or high (> 80 %) virus incidence (Nelson et al. 1992). Thus, the logistic face of epidemics can be emphasized (Mora-Aguilera and Webb, 1993). A modified version of

two-dimensional distance class analysis was used by Nelson et al. (1993) to characterize epidemics in clover of several pathogens including one potyvirus, and by Mora-Aguilera and Webb (1993) in a preliminary study of spatial patterns of WMV-2 epidemics in watermelon.

Other methods, such as autocorrelation, geostatistics, and the beta-binomial distribution, have been applied to describe spatial patterns of several pathosystems (Hughes and Madden, 1993; Gottwald et al., 1992; Chellemi et al., 1991; Lecoustre et al., 1989; Reynolds and Madden, 1988). These are additional techniques that can be used, preferentially combined with other approaches, to study spatial patterns of virus epidemics. Regardless of the fact that several analytical methods have been proposed to study spatial patterns and dispersal of pathogens (Table 2.6), the application of these methods to plant virus epidemics is not common, and just how spatial patterns are affected by the behavior of aphid vectors still remains poorly understood.

Forecasting of Nonpersistent Virus Epidemics

Forecasting plant disease epidemics in theory has the final purpose of assisting in the design of efficient programs of pest control (Campbell and Madden, 1990; Madden and Ellis, 1988; Thresh, 1986). Forecasting of nonpersistent virus epidemics departs, in practice, from this view. Two reasons support this statement. There is not a single method of control which gives predictable results (Webb and Linda, 1994; Marco, 1993; Perring and Farrar, 1993; Hull and Davies, 1992; Hunter and Ullman, 1992; Kucharek and Purcifull, 1989; Jones, 1987; Maelzer, 1986; Raccah, 1986; Harpaz, 1982; Adlerz, and Everett, 1968). Most nonpersistent virus epidemics reach 100% incidence at some point during the growing season, whereupon no prediction is needed to determine this outcome (Webb and Linda, 1994; Hander et al., 1993; Dahal, 1993; Castle, 1992; Mora-Aguilera et al., 1992; 1995; Lecoq, 1991b; Madden et al., 1987a;

1987c; Gray, 1986), and viral epidemics vectored by aphids are usually determined by macroclimatic events, sometimes far beyond the field surroundings (Berger and Ferris, 1989; Taylor, 1986).

Successful forecasting of pathogens other than viruses rely heavily on variables monitored at the microclimatic level (canopy, phylloplane, or rhizosphere), and control tactics are based on the availability of pesticides with various modes of action (Madden and Campbell, 1990; Madden and Ellis, 1988; Chuang and Jeger, 1987; Coakley et al., 1985). Thus, the application of forecasting to plant virus epidemics has to be approached with different perspectives and expectations. That is, the identification, selection, and evaluation of variables in the development of epidemics are primary objectives. Although this may eventually lead to the generation or an optimization of available methods of control, no immediate application can necessarily be expected (Mora-Aguilera et al., 1993b; Raccah et al., 1988; Thresh, 1986; Sigvald, 1986; Ruesink and Irwin, 1986; Marcus and Raccah, 1986). Epidemics of persistently transmitted viruses may depart from this view because of possibilities for chemical control as pointed out by Plumb et al. (1986) and Heathcote (1986).

Forecasting of plant virus epidemics can be thought of as an attempt to understand, through a mathematical approach, a pathosystem at the agroecosystem level.

Forecasting Approaches in Virus Epidemics

Transitory, noncolonizing as well as colonizing aphid species play an important role in the development of most virus epidemics. These aphid species may arrive from local or long distance sources (Mora-Aguilera et al., 1993b; 1992; Madden et al., 1987c; Taylor, 1986). Most modeling studies have been carried out to find the association of vectors with changes of viral disease incidence. Although there are

some reports where relationships between vector dynamics and progress of virus incidence have not been found (Basky 1986, Toba et al., 1977), such relationships are generally well documented (Madden et al., 1990; Clement et al., 1986, Sigvald 1986). Two general views used in forecasting of virus epidemics will be presented: the stochastic and the deterministic approach. Since only a few examples of forecasting virus epidemics are available in the literature, this discussion will not be restricted to potyviruses.

Multiple Regression Models: a Stochastic Approach

Regression analysis, particularly multiple regression models, has been used to model epidemics of plant viruses using changes in viral disease incidence as the dependent variable and each vector species as an independent variable. Positive associations between these two components have been found in studies in which lag time adjustments were considered (Mora-Aguilera et al., 1993b; Raccah et al., 1988). Lag time adjustments can be explained biologically as the average incubation time required for viral symptom expression after inoculation. The main objective in most studies of this type is to define the most important vector species, rather than support a specific control program (Mora-Aguilera et al., 1993a; Plumb et al., 1986; Raccah et al., 1988; Madden et al., 1983).

Two basic biological assumptions underlay the application of multiple regression models to forecasting of virus epidemics. Because of the statistical meaning of parameters within the model (βi, where $i = 1$ to n-variables in the model), and the aphid type taken into account in modeling (counts of alate, transient aphids), it is necessary to recognize that only epidemics driven by noncolonizing alate species, and nonpersistently transmitted viruses may be suitable for use in multiple regression models. Epidemics of PRSV-P in *Carica papaya* have these characteristics (Mora-Aguilera et al., 1992), and a model was developed and validated using 60 PRSV-P

epidemics over a five year period (Mora-Aguilera et al., 1993b). In that study, counts of *A. nerii (An)* and *A. gossypii (Ag)* lagged in time and the interaction of speed and wind duration (*W*) with precipitation (*P*), predicted changes of disease incidence ($\hat{y}$) in 40% ($R^2 \geq 60\%$) of the epidemics (24 out of 60). The forecasting model selected after a validation process was:

$$\hat{y} = 0.442 + 0.168\ An + 0.658\ Ag + 0.000092\ PW$$

Multiple regression models have been used in systems where the basic assumptions are not fully satisfied. Madden et al. (1983), and Raccah et al. (1988), used regression models to identify important aphid species in the spread of maize dwarf mosaic virus (MDMV) in *Zea mays*, and tobacco etch (TEV) and tobacco vein mottling (TVMV) viruses in *Nicotiana tabacum*. Although the first assumption was fulfilled (nonpersistently transmissible viruses), the second assumption (alate, noncolonizer aphids) was not satisfied.

Aphid vectors of MDMV, TEV, and TVMV are able to colonize maize and tobacco (Madden 1993, personal communication), which means that it is not possible to separate specimens that have immigrated to the field prior to being caught in the trap from those that are moving within the field. Additionally, the possibility of colonization (Madden et al., 1983; Raccah et al., 1988) implies that secondary virus dispersion by apterous aphids is not considered in the regression models.

Even if biological assumptions are appropriate for use of multiple regression analysis, this approach has two major drawbacks. One is that the method may fail to provide specific biological explanations regarding interactions in the host-virus-vector(s) system, and another is that important variables could be eliminated. Reliability of the method regarding these two problems is determined by the model structure.

Because the selection of variables and determination of parameters is based on quantitative associations between disease intensity and aphid species, environmental and biological factors such as incubation period, effect of host genotype on transmission, transmission rates, serial transmission, etc. could be masked in the process. For example, each model parameter can be thought of as an average transmission rate under natural conditions. Thus, it is an estimation of *vector propensity* (*sensu* Irwin and Ruesink, 1986). The combination of such parameters with counts of their respective vector species, can be viewed as an estimation of *vector intensity* (*sensu* Irwin and Ruesink, 1986), and the lag factor(s) can be conceived of as average incubation period(s). It should be noted that parameters are constant values within the model and are used to estimate average changes in a dynamic system. Consequently the estimation of biological processes is only an approximation.

The selection process of independent variables in multiple regression analysis is used to identify potential vector species from a particular data set. Thus, it is possible that under different conditions, vectors not selected in the original model may be important in virus dispersal (Madden et al., 1983).

In summary, it is possible to use multiple regression models to study the relationship between a nonpersistently transmitted virus disease and its vectors provided the absence of colonization. Many potyviruses, including WMV-2, PRSV-W, ZYMV, PRSV-W, and other viruses share these characteristics (Webb and Kok-Yokomi, 1993; Adlerz, 1987; 1978b; 1974a; Halbert et al., 1981; Van Harten, 1983; Mora-Aguilera et al., 1992).

Regression models, applied correctly, can be used when a general description of the structure of an epidemic is required or when exploratory data is needed to develop a simulation model (Irwin and Ruesink, 1986; Watson and Healy, 1953). However, the actual success of this approach is contingent upon the level of understanding of the basic components of the pathosystem, including regional or local

knowledge of the abundance and species composition of aphid vectors, the presence of colonizing species, sources of inoculum, and type of transmission.

Simulation Models: a Deterministic Approach

Simulation modeling has been used to assess the dynamics of virus diseases under field conditions and to predict the development of epidemics (Madden et al., 1990; Sigvald, 1986; Marcus and Raccah, 1986; Ruesink and Irwin,1986; Watson and Healy, 1953).

As in multiple regression models, simulation models may take several vector species into account. However, simulation models may also take into account changes in virus acquisition and inoculation periods and the effects of the host phenology on the vector (Ruesink and Irwin, 1986; Marcus and Raccah, 1986; Watson and Healy, 1953).

The first simulation model of a virus disease was developed by Watson and Healy (1953) for semipersistent viruses transmitted by aphids in sugar beet (*Beta* sp). Recently, this approach has also been applied to nonpersistent aphid-borne viruses (Ruesink and Irwin, 1986; Marcus and Raccah, 1986).

Ruesink and Irwin (1986) predicted soybean mosaic virus incidence on a given day *(DY)* with the model:

$$DY = H [\, 1 - exp (- I_n / P) \,]$$

in which H is the number of healthy plants at a given time period, P is the total number of plants, and I_n is the number of inoculations and/or transmissions, estimated by:

$$I_n = p, \Sigma\, p_z\, (i)\, T\, (i)$$

where p_1 is the probability that an aphid came directly from a source plant (a factor which can be the proportion of infected plants at a given time period); $p_2(i)$ is the probability of a successful inoculation by an aphid of species *i* when it moves from a source plant to a healthy plant; and $T(i)$ is the trap catch for group *i* in a period of time. Estimates of $p_2(i)$ were done using field live assays. The p_2-values for each species was a fixed parameter through the growth season (Ruesink and Irwin, 1986).

Marcus and Raccah (1986) developed a more generalized model by including aphids from within and outside the field. In this model, the increase in disease incidence is given by:

$$DY = (1-y_i)\,[\,1-exp\,(\,-a_1 - R_2\,y_i)\,]$$

in which y_i is the proportion of currently infected plants, so that $(1-y_i)$ is defined as H from the proposed model by Ruesink and Irwin (1986). Similarly, R_2 is analogous to I_n of that model. R_2 can therefore be defined as the number of inoculations per plant by internal aphids that had access to a single source plant and a_1 defined as the number of inoculations per plant by external aphids.

Simulation models are especially important for understanding the causal mechanisms involved in the build-up of a particular epidemic. Therefore, deterministic approaches are usually used for this type of modeling, and intensive research has to be done to assess and determine specific parameter values to be used in the models (Schultz et al., 1983; Irwin and Ruesink, 1986).

From a pragmatic point of view, simulation models could be more expensive to develop, and the accuracy of forecasting could be similar to stochastic models, such as linear or nonlinear regression models, and empirical forecasting systems (Mora-Aguilera et al., 1993b; Madden et al., 1990; Raccah et al., 1988; Madden et al., 1983; Madden and Ellis, 1988).

The Problem of Multidimensionality in Modeling Virus Epidemics

An epidemiological event is by nature a multivariate process. In order to understand and fully characterize the development of virus epidemics, several variables involving the environment, host plant, vectors, virus, and the human component need to be measured.

The use of multivariate tools is essential for modeling epidemiologic processes. However, the gain in explanatory capacity provided by a multivariate procedure may be offset by the presence of multicollinearity, i.e., the presence of variable(s) highly correlated to other variable(s) in the data set. Consequently, multicollinearity can reduce the robustness or reliability of inferences derived from a multivariate statistical analysis (Jolliffe, 1986; Hawkins and Fatti, 1984).

Multiple regression analysis (which can be viewed as a particular case of canonical correlation), frequently used in correlative studies between virus incidence and vector density (Mora-Aguilera et al., 1993b; Raccah et al., 1988; Madden et al., 1983), is often affected by the multicollinearity problem. Multicollinearity can be detected within multiple regresion models by computing specific statistics such as VIF (variance inflation factor), but it can not be corrected (Mora-Aguilera et al., 1993b; Chuang and Jeger, 1987). Therefore approaches to identify and correct or reduce multicollinearity are fundamental in forecasting plant virus epidemics with stochastic models.

Principal component analysis (PCA) and biplot displays are two multivariate techniques which can be use to reduce and eventually eliminate multicollinearity and spurious variables, a process referred to as reduction of dimensionality. Principal component analysis and biplot displays can also be used for outlier detection and clustering of observations of orthogonal variables (Mora-Aguilera et al. 1993a; 1995; Anderson et al., 1990).

Principal Component Analysis

Principal component analysis is a multivariate statistical technique that transforms an original set of variables into a new set of variables, called principal components, which are uncorrelated. The first few new variables retain most of the information from the original data. The main goal of PCA is to reduce dimensionality of the original data (Jolliffe, 1986).

Principal component analysis achieves the reduction of dimensionality by weighting each variable and then adding the products for each observation in a linear combination. The resultant values constitute the new variable, the principal component (PC). This linear combination can be represented as:

$$u_n = a_1 x_1 + a_2 x_2 + \ldots + a_p x_p$$

where u is the value or score of the new variable or principal component for the n^{th} observation; $x_1, x_2, \ldots x_p$ are the values of p-variables having an overall p-dimension; and $a_1, a_2, \ldots, a_p$ are variable weights (coefficients) which are specific for each variable. In terms of matrix algebra, the whole set of weights is called an eigenvector, which is unique to each principal component. In this example, p-dimensional data was reduced to one-dimensional data.

The new set of variables or principal components have the following important characteristics (Hatcher, 1994; Jolliffe, 1986; Hawkins and Fatti, 1984):

- the maximum number of components that can be generated is equal to the total number of original variables (i.e., p-components).
- the first principal component is constructed to capture the largest amount of information or variation in the original variables, the second principal component has the second highest variance, and so on.

- the variance explained for each principal component is expressed in a numerical value called eigenvalue (denoted as λ).
- the principal components generated are uncorrelated or orthogonal; and
- the final number of principal components selected determines the new dimensionality of the data set.

The desired amount of reduction of dimensionality is not always clear. Two criteria often used to determine the most important principal components are the selection of those components associated with large partial variances ($\lambda \geq 0.70$ or ≥ 1.0) and the selection of those components with cumulative partial variances of 70-90% (Jolliffe, 1986; Hawkins and Fatti, 1984).

Although the principal components can be used to characterize the original data, original variables can be used for the same purpose. This approach implies the identification of variables that contribute most to the variation of the selected principal components. This method departs from examples found in plant disease epidemiology (Anderson et al., 1990; Madden and Pennypacker, 1979), and in plant pathology in general, but is well supported in the statistical literature (Hawkins and Fatti, 1984; Jolliffe, 1986).

Biplot Displays

A simple definition of biplot in the framework of PCA is simply a graphical display of *p*-variables in a two-dimensional representation. There are different types of biplot displays depending on the power value applied to a system of matrices referred to as singular value decomposition (SVD) (Jolliffe, 1986). The simplest type of biplot, which is obtained with a power of zero, has been shown to be adequate for multivariate temporal data (Anderson et al., 1990; Mora-Aguilera et al., 1995).

A biplot is generated by plotting *p*-variables onto a pair of principal components in a coordinate system (Gabriel, 1971). For example, if the first and second principal component are selected, then the loadings of the eigenvectors associated with those principal components and with a particular variable are selected and plotted onto the coordinate system. Vectors are then constructed by projecting a line from those plotted variables to the origin of the coordinate system. The longest and more parallel vectors to the axis comprise most of the data variability in the pair of principal components plotted (Mora-Aguilera and Campbell, 1995; Hatcher, 1994; Jolliffe, 1986).

Rotation of Principal Components

A procedure that plays a fundamental role in PCA and biplot displays is the rotation of principal components (Jolliffe, 1989). Rotation is used to increase the interpretability of the principal components by determining the importance of individual variables in a particular principal component. This is achieved by shifting the orientation of the principal components in multivariate space. The joint variance accounted for by rotated-PCs is still equal to the unrotated ones, but their individual variances are more or less evenly spread among the PCs. Therefore, rotation should be done on groups of principal components with similar variance, i.e. those comprising most of the variability in the data (Jolliffe, 1989).

Because of the exploratory capabilities of principal component analysis and biplot displays of varimax rotated major axis, these analytical tools may have great potential in stochastic modeling of virus epidemics. These multivariate approaches are compatible with the multifactorial structure of epidemics and can be thought of as the initial stage of virus disease modeling. Yet, its application to plant virus epidemics has been in the context of comparative epidemiology (Mora-Aguilera et al., 1995; Madden and Pennypacker, 1979; Campbell et al., 1980). Its value in forecasting of viral disease

still needs to be demonstrated. Discriminate analysis, another multivariate technique, combined with stepwise regression has been successfully used in predicting maize dwarf mosaic in maize (*Zea mays*) (Madden, 1983). It is possible that analytical tools, other than classical linear or nonlinear models, may be used in forecasting stochastic models. This constitutes an open field of methodological research in plant epidemiology.

CHAPTER 3
TEMPORAL SPREAD OF TWO POTYVIRUSES IN WATERMELON

Introduction

Watermelon (*Citrullus lanatus*) is the most extensively grown cucurbit crop in Florida with more than 40 thousand acres planted annually from 1989 to 1993, followed by cucumber (*Cucumis sativus*) and squash (*Cucurbita pepo*) with more than 15, 000 and 12, 000 acres, respectively (Anonymous, 1994). Watermelon is grown in at least 30 of the 67 Florida counties, with the highest harvested acreage in the northern region (Anonymous, 1994).

Several factors, including weather, marketability, and pests have significantly impacted the watermelon industry in Florida for several decades. Among the plant pathogens, watermelon mosaic virus 2 (WMV-2), papaya ringspot virus type W (PRSV-W), and zucchini yellow mosaic virus (ZYMV) are the most important viruses affecting production of watermelon and other cucurbits in the state (Webb and Linda, 1993; Kucharek and Purcifull, 1989; Purcifull et al., 1988; 1984a; Adlerz et al., 1983; 1978a; 1969).

Temporal spread of WMV-2, PRSV-W, and ZYMV in cucurbit crops has been studied in the United States in Florida (Adlerz, 1974a; 1978a; Webb and Linda, 1993), North Carolina (Gray et al., 1986), Arkansas (Hander et al., 1993), Arizona (Nelson and Tuttle, 1969) and California (Castle, 1992; Perring et al., 1989); and in other countries such as Nepal (Dahal, 1992), France (Lecoq et al., 1991b) and Taiwan (Wang et al., 1991).

In North Carolina, Gray et al. (1986) reported seasonal differences in epidemics induced by WMV-2 in three genotypes of *Cucumis melo*. Average apparent infection rates of 0.027-0.049 units $days^{-1}$, high final disease incidences between 92%-100% which were reached in about 35 days, and no differences in epidemic progress curves among genotypes were detected in summer plantings. In contrast, low infection rates (0.0033-0.0358 units day^{-1}), low final disease incidences (11% - 65%), and differences in epidemic progress curves among genotypes were found in spring plantings.

Seasonal and regional effects on epidemic development were also reported by Hander et al. (1993). Final disease incidences of 23.2% (WMV-2) and 92.8% (PRSV-W) were found in late summer squash plantings in south central and western Arkansas, respectively. No plants infected with WMV-2, ZYMV, and PRSV-W were detected in spring plantings.

In southwestern Arizona, Nelson and Tuttle (1969) characterized spring epidemics of WMV-2 and cucumber mosaic cucumovirus (CMV) in *C. melo*. Five epidemics, associated with independent years, had final disease incidences in the range of 75 to 100% and the duration of epidemics ranged from 35 to 60 days. All five epidemics exhibited typical sigmoidal disease progress curves. Two other epidemics reached 20 and 40% incidence.

In southern California, spread of ZYMV and WMV-2 in spring muskmelon fields was reported by Castle (1992). Although WMV-2 spread faster than ZYMV in three out of five fields, typical logistic sigmoidal curves and 100% final disease incidences were observed in independently monitored epidemics of WMV-2 and ZYMV. Total epidemic time ranged from 10 to 46 days. In France, opposite results were reported by Lecoq et al. (1991b) in a two-year study. In the latter study, although both viruses reached 100 % incidence in 42-49 days, ZYMV spread faster than WMV-2.

In previous surveys in Florida, WMV-2 and PRSV-W were the most common viruses detected in watermelon and squash production in Central and South Florida,

respectively (Webb and Linda, 1993; Adlerz et al., 1983; Purcifull et al., 1986; Purcifull et al., 1984).

In contrast to early findings (Adlerz, 1978a), epidemic outbreaks and serious losses due to WMV-2 have been detected since 1988 in Central Florida (S. E. Webb, personal communication). In addition, ZYMV prevalence may have increased as suggested by a study recently conducted from 1989-1991 (Webb and Linda, 1993).

In this research, the temporal spread of spring viral epidemics, the most likely to be driven by limited inoculum sources in Central Florida, was characterized to provide epidemiological criteria for future implementation of control programs. In addition, the composition and relative importance of potyviruses infecting watermelon, including the current status of ZYMV, was determined using serological techniques.

Materials and Methods

Establishment of field plantings. In 1993 and 1994 four experimental plots were established in a 6-ha field at the Central Florida Research and Education Center at Leesburg, FL. Each plot consisted of 15 east-west oriented rows 30 m long, spaced 3 m apart with plants separated by 1.5 m within rows. This spacing adheres to Florida recommendations for commercial production of large fruit (Hochmuth and Elmstrom, 1992). Roadways perpendicular to the direction of rows were made every 7.6 m to allow application of fungicides. Plots were surrounded by 4 m bare soil and separated by 60 m of bahia grass pasture east-west and 30 m north-south from each other. Plots were nearly level on a well-drained sandy soil. Plot sites had not been planted with watermelon within the past five years to prevent fusarium wilt (*Fusarium oxysporum* f.sp. *niveum*). On 24 March (1993) and 14 March (1994), the cultivar Fiesta was directly seeded onto raised beds at a rate of 3-4 seeds per hill. A total of 300 plants per plot were left after thinning the rows to one plant per hill, 20 per row, on 16 April

(1993) and 22 April (1994). To reduce wind damage and sand-blasting of young seedlings, windbreaks of rye were established between every other row in late January and removed on 21 April in both years. In 1993, a series of experimental fields of several cucurbits were located west and southwest of the watermelon plots. A vineyard (*Vitis* spp.) was located east of the plots. In 1994, an experimental field of about 1.5 ha, used in watermelon breeding trials, was located south of the plots A and B.

Fertilizer was applied four times: at preplanting (6-8-8, 897 kg/ha) on 8 March; at emergence (15-0-14, 112 kg/ha) on 8 April (1993) and 24 March (1994); as a side-dressing (15-0-14, 224 kg/ha) on 21 April (1993) and 14 April (1994); at lay-by (15-0-14, 336 kg/ha) on 28 April (1993) and 21 April (1994). The following pesticides were applied: sodium salt of naptalm benzoate 23.7% (4-l /378.5-l, herbicide) 23 March (1993), 15 March (1994); permethrin 3.2 EC (10-ml /94.6-l, insecticide) 8 April (1994); mancozeb (1-1.5 l /378.5-l, fungicide) 14 and 21 May (1993), 6, 13, and 27 May (1994); and chlorothalonil (1-1.5 l / 378.5-l, fungicide) 4 June (1993), 20 May (1994). A total of 18 cm of water per season were provided by 10 weekly irrigations. Overhead sprinklers at operating times of 45 min and 90 psi pressure were used from 1 April to 3 June (1993) and from 21 March to 24 May (1994).

Disease assessment. Each of the 300 plants per plot was observed once every 3 days from emergence on 29 March (1993) and 19 March (1994) to harvesting on 24 June (1993) and 2 June (1994) for symptoms of viral infection. Typical symptoms included distortion of leaves and ends of runners, and vein-banding, green spotting, mottle, vein-clearing chlorosis or moderate necrosis of leaves. No attempt was made to use these symptoms to identify any of the viruses previously found infecting watermelon in Florida because of the great biological variability of these potyviruses and a high possibility of mixed infections (Webb and Linda, 1993; Lecoq and Purcifull, 1992; Adlerz et al., 1983). The location of each individual diseased plant was recorded for each plot and evaluation date.

Phenological assessments and harvest. In 1993, about 10 randomly selected plants per plot were inspected every three days for flowering, fruit set and development, number of runners (vines) and percentage of total ground covered with watermelon foliage. In 1994, 39 plants randomly selected from plots A, B and D were marked and evaluated every three days. The number of flowers, length of first runner, and number, diameter, and length of fruits per plant were recorded until 19 May (62 days after planting). After this date, evaluations included only estimated percentage of ground coverage.

A total of 279 plants from plot C on 17 and 18 June in 1993, and 1190 plants from the four plots on 9 to 14 June in 1994 were harvested and measured independently. Prior to harvest, each fruit and the vine to which it was attached were tagged to keep track of the plant that produced them. The time of harvest, as well as the selection of fruits to be harvested were always defined by Harvey Beasley, an experienced worker in watermelon production. Fruit weight and soluble solids per fruit were measured at the field the same day that the fruit were harvested.

Serological tests. Because WMV-2, ZYMV, and PRSV-W are considered the most important and common viruses in watermelon in Florida (Webb and Linda, 1993; Purcifull et al., 1988; Purcifull et al., 1984a; Adlerz et al., 1983), enzyme-linked immunosorbent assays (ELISA) were conducted to determine presence/absence of these viruses as described by Clark and Adams (1977).

A γ-globulin (IgG) purification and IgG enzyme conjugation were completed for the polyclonal antisera 1134 (WMV-2), 1142 (PRSV-W), and 1160 (ZYMV). The optical densities at 280 nm ($O.D._{280nm}$) for the purified IgG fractions of the antisera were, respectively, 1.409, 1.426, and 1.344. Concentrations of IgG were estimated using the formula: 1.4 O.D. units ≡ 1 mg / ml. Optimization of coating and enzyme-labeled IgG concentrations were determined following the method of Clark and Adams (1977). Alkaline-phosphatase type VII - Sigma P5521 was used for enzyme conjugation.

Plant sap (1:10 w/v) was extracted by pressing leaf tissue within 15 X 10 cm zip plastic bags using an electrical rotor (MAXI-TORK™ 42522, Dayton Electric Co. Chicago 60648, USA). ELISA results were evaluated as colorimetric responses by determining the absorbance at 405 nm with a microtiter plate reader (MICROPLATE EL309, Bio-Tek instruments).

The presence or absence of WMV-2, ZYMV, PRSV-W, squash mosaic virus, a possible potexvirus from *Trichosanthes* (Purcifull et al., 1988), cucumber mosaic virus, and an unnamed potyvirus (coded as 2932) previously found in cucurbits in Florida (Purcifull et al., 1991), were also determined in a small number of samples with sodium dodecyl sulfate (SDS)-immunodiffusion tests (Purcifull et al., 1984a; Purcifull and Batchelor, 1977). The SDS-immunodiffusion tests were conducted in media containing 0.8% Noble agar, 0.5% SDS, and 1.0% sodium azide. Crude extracts were heated in a boiling water bath for 4 min before use (Purcifull et al. 1981). A complete list of antisera used in serological tests is given in Table 3.1.

Table 3.1. Antisera used in ELISA and SDS-immunodiffusion assays in watermelon (*Citrullus lanutus*) crop.

Name of virus	Abbreviation of virus	Antiserum number	[1]Antiserum prepared to	[2]Antiserum used for
Cucumber mosaic	CMV	965	SDS-V	ID
Papaya ringspot	PRSV-W	1125	CP	ID
Papaya ringspot	PRSV-W	1142	V	ELISA
Squash mosaic	SMV	876	SDS-V	ID
[3]Trichosanthes virus	TV	1129	SDS-V	ID
[4]Unnamed	2932	1163	CP	ID
Watermelon mosaic	WMV-2	1134	V	ELISA, ID
Zucchini yellow mosaic	ZYMV	1133	CP	ID
Zucchini yellow mosaic	ZYMV	1160	CP	ELISA

[1] SDS-V = SDS-treated virus; CP = capsid protein; V = untreated virus.
[2] ELISA = Enzyme-linked immunosorbent assay; ID = SDS - Immunodiffusion.
[3] Possible potexvirus as reported previously (Purcifull et al., 1988).
[4] Potyvirus serologically different from PRSV-W, WMV-2, and ZYMV (Purcifull et al., 1991).

Sampling. All plants of one plot in 1993 and 1994 were individually sampled on 7 June (1993), and 26 May (1994) for testing by ELISA. A total of 294 and 298 plants were sampled in 1993 and 1994, respectively. The end sections opposite to the petioles of 10-15 fully expanded leaves were selected from primary or secondary runners showing clear or putative viral symptoms. When showing symptoms, the first runner (i.e. the longest and thickest stem) was always selected for sampling. Efforts were made to obtain a sample representative of the range of symptoms in the runner selected. During field sampling, bagged samples were stored in a portable cooler to prevent dehydration.

To detect viruses with SDS-immunodiffusion in 1993, two plants per plot were selected, based on symptoms, on 28 June. In 1994, the first infected plants per plot on 2 May were sampled. Thus, three plants in plot A, four in plot B, and one in plot C were sampled. Sampling per plant was done as previously described except that complete leaves were taken and bagged. In 1994, three fields from Marion county and one from Lake county were inspected for virus infection on 18 May and 21 May, respectively. From a total of 26 infected plants, four plants were selected, based on symptoms, for SDS-immunodiffusion as described previously.

Temporal analysis. Disease progress was analyzed for each plot separately. The starting point of each epidemic was determined to be when the first infected plant was detected in each plot. Thus, a variable number of observations were included in the analysis.

The linearized form of the logistic [$\ln (y/(1 - y)) = \ln(y_o/(1 - y_o) + r_L t)$], Gompertz [$-\ln(-\ln y) = -\ln(-\ln y_o) + r_G t$], and monomolecular model [$\ln (1/(1 - y)) = \ln(1/(1-y_o)) + r_M t$] were evaluated for appropriateness to describe disease incidence with time (Campbell and Madden, 1990).

Model parameters were estimated with the least squares technique of simple linear regression using the SAS procedure PROC GLM (SAS, 1988). Appropriateness

of a given model was evaluated based on the coefficient of determination (r^2), and plot of the standardized residuals vs. predicted values. Average apparent infection rates within and between years were compared using the paired *t*-test method (Campbell and Madden, 1990).

Yield loss modeling. Statistical correlations of fruit weight as well as fruit sugar content per individual plant against the number of days that such plant remained healthy were performed with simple linear and quadratic regression models with independent data sets for each year. Model parameters were estimated with the least squares technique of simple linear regression using the SAS procedure PROC GLM (SAS, 1988). Appropriateness of a given model was evaluated based on the coefficient of determination (r^2).

Disease gradients analysis. In 1994, incidence gradients were detected in plots A and B toward a nearby experimental watermelon field of 2.3 ha. Twenty-three percent of this field was transplanted on 14 March. Some of these plants may have been infected with virus at the time of transplanting because virus infection was detected in the greenhouse in which the transplants were produced (D. L. Hopkins and G. W. Elmstrom, personal communication). The nature of the virus was not serologically determined. The field was located 40 m from the south edge of plots A and B.

To evaluate this possible source on the development of epidemics, dispersal gradients were determined with the solution equation of the nonstationary wave model (without the advection term) as described by Murray (1989, eqn. 9.21, p. 238-329) and Okubo (1980, eqn. 6.22, p. 99).

The one-dimensional version of this model was used because of the unidirectionality of gradients. The nonstationary wave model was selected because of its flexibility to fit a series of gradients with curves of different shapes. In addition, the parameters can be interpreted biologically.

The model is an extension of the classical diffusion equation in which three basic characteristics applied (Okubo, 1980): 1) a dispersive force associated with random movement; 2) an attractive force, which induces directed movement of individuals toward favorable environments; and 3) a population pressure due to interference between individuals. The model can be written as:

$$y(d,t) = n_0 [\lambda(t)]^{-1} \left[1 - \left\{ \frac{d}{r_0 \lambda(t)} \right\}^2 \right]^{1/m} \quad , |d| \le r_0 \lambda(t)$$

where,

$$\lambda(t) = \left(\frac{t}{t_0} \right)^{1/(2+m)} \quad , \quad r_0 = \frac{Q\Gamma\left(\frac{1}{m} + \frac{3}{2}\right)}{\left\{ \pi^{1/2} n_0 \Gamma\left(\frac{1}{m} + 1\right) \right\}} \quad , \quad t_0 = \frac{{r_0}^2 m}{2D_0 (m+2)}$$

where, y is the percentage of disease incidence at the distance d from the viral source at time t after the viral source became infectious. Γ is the gamma function and Q can be viewed as an estimator of the total number of individual aphid vectors that successfully acquired the virus at the source.

Because the viral source was a naturally infected field, the number of infectious plants from that field increased with time and so did the number of aphids that successfully acquired the virus. Thus, Q was not held constant for the different evaluation dates. Also, because of the variability of Q, gradients were estimated with the parameter $t = 1$. D_0, m, and n_0 are positive, constant parameters.

D_0 is the diffusivity parameter which tends to decrease as the border effect appears. The m is a shape parameter of the gradient. With higher m-values ($m > 1$), the gradient shows a platykurtic distribution (Okubo, 1980) which may indicate dispersion due to high population pressure of aphid vectors at the source. Small,

positive m-values ($m < 1$) are associated with a leptokurtic distribution (Okubo, 1980), which may indicate a border effect of infected plants due to secondary dispersion of aphid vectors. The n_0 is a parameter of reference of the population density.

In addition to the nonstationary wave model, two empirical models, the power law [ln (y) = ln (a) - b ln (x), y = estimated disease incidence at distance x_i from the source] and exponential model [ln (y) = ln (a) - bx] were used for comparative purposes (Campbell and Madden, 1990; McCartney and Fitt, 1985).

Data sets of three evaluation dates for plot A and four for plot B, were evaluated independently. At each evaluation date, accumulated disease incidence of the first three rows and two rows thereafter for plot A and every two rows for plot B were used in the analysis. Distances were estimated starting with the 40 m of separation between the plot serving as the source of the virus and the plots in this experiment.

Because visual inspection of plotted percentage of disease incidence against distance and preliminary analysis results suggested a light edge effect on the north edge of each plot, the last three rows were eliminated from the analysis.

A nonderivative approach (DUD) subroutine of PROC NLIN of SAS (1988) was used to analyze each data set with the nonstationary wave model. This approach was selected to avoid calculations of partial differential equations for at least three parameters (Appendix 2).

Convergence criterion, sum square of residuals and plot of residuals vs. distance were inspected to evaluate the stability of the model. PROC GLM of SAS (1988) was integrated in the analysis to determine the degree of association between predicted and actual observations. The r-square value of the linear regression and its statistical significance were used as estimations of goodness of fit.

Parameters of the power law and exponential models were estimated with the least squares technique of simple linear regression using PROC GLM of SAS (1988) (Appendix 2).

Results

Temporal Dispersion. For the watermelon plots in 1993 and 1994, progress of spring virus epidemics followed symmetrically sigmoid curves (Fig. 3.1A-B).

In 1993, the first plant showing viral symptoms was recorded on 30 April in plot C, 40 days after planting. The first infected plants in the remaining plots were detected at 54 (plots A and D) and 61 days (plot B) after planting. The total epidemic duration time ranged from 36 to 51 days, reaching accumulated disease incidences of 93-98% on 23 and 29 June, 91 to 97 days after planting. The 50% accumulated disease incidence was reached within the range of 70 to 78 days after planting, 16 to 30 days after the initial diseased plants were recorded (Table 3.2).

Table 3.2. Curve parameters associated with potyvirus epidemics in watermelon grown during the spring season of 1993 and 1994.

Year / Plot[1]	Yo [2]	Y_f	Xo	T_{50}	T_t	k_L
1993-A	0.003	0.98	54	19	43	0.225
1993-B	0.003	0.98	61	16	36	0.225
1993-C	0.003	0.93	40	30	51	0.193
1993-D	0.003	0.98	54	24	43	0.222
1994-A	0.003	1.00	41	15	28	0.409
1994-B	0.006	1.00	41	15	28	0.393
1994-C	0.016	1.00	48	09	28	0.362
1994-D	0.003	1.00	44	16	35	0.320

[1] WMV-2 in 1993; Mostly ZYMV and WMV-2 in 1994.
[2] Curve parameters: Yo = proportion of initial disease incidence; Y_f = proportion of final disease incidence; Xo = time in days from planting to Yo; T_{50} = time in days from Yo to 50% incidence; T_t = epidemic duration time in days; k_L = average apparent infection rate estimated with the linearized logistic model.

In 1994, the first plants showing viral symptoms were recorded on 25 April in plot A and B, 41 days after planting. The first infected plants in the remaining plots were detected at 44 (plot D) and 48 days (plot C) after planting. The total epidemic duration time ranged from 28 to 35 days, reaching accumulated disease incidences of

100% on 26 May and 2 June, 69 to 76 days after planting. The 50% accumulated disease incidence was reached within the range of 56 to 60 days after planting, 9 to 16 days after the initial diseased plants were recorded (Table 3.2).

In 1993, fruit set, a phenological stage of five runners (vines), and 50% of ground covered by folliage were reached before the onset of epidemics (Figure 3.1A). In contrast, in 1994, epidemic onset occurred before flowering, a phenological stage of three to four runners (vines), and 15% of ground covered by watermelon folliage (Figure 3.1B).

In 1993, at harvest on 17 June (85 days after planting), the accumulated disease incidence ranged from 83 to 91%, whereas in 1994, epidemic curves were in the asymptotic phase when harvesting was done on 9 - 14 June (83 to 88 days after planting). Spring epidemics in 1993 and 1994 were best described by the logistic model based on the percentage of variance explained (r-square values), and examination of residuals plots.

Average apparent infection rates estimated with the logistic model ranged from 0.193 to 0.225 units day^{-1} in 1993 and from 0.320-0.409 units day^{-1} in 1994. The r-square values associated with this model were higher than 0.93 ($p \leq 0.05$) for the two years (Table 3.3).

The average apparent infection rates were compared within and between the two years by the paired *t*-test (Table 3.4). With the exception of the rate of plot C in 1993, which was significantly different (P = 0.05) from those of plot A and D (t_{est} =2.253 and 2.086, respectively), and of the rate of plot D in 1994, which was significantly different (P = 0.05) from that of plot A (t_{est} = 2.645), epidemic rates were statistically similar within years. In contrast, all paired comparisons between the two years were statistically different (Table 3.4). Thus, epidemics within a year were homogeneous but differed among the two seasons (i.e., 1993 and 1994).

Table 3.3. Linearized model fitted to potyvirus epidemics in watermelon grown during the spring season of 1993 and 1994. Bold numbers are associated wiht the best fitted model.

Year / Plot	Y_f[1]	Model[2]	r^2	std[3]	Epidemic Rate[4]
1993-A	0.98	L	**0.98**	**0.009**	**0.225**
		G	0.98	0.006	0.148
		M	0.91	0.010	0.108
1993-B	0.98	L	**0.94**	**0.017**	**0.225**
		G	0.96	0.009	0.149
		M	0.89	0.012	0.108
1993-C	0.93	L	**0.96**	**0.011**	**0.193**
		G	0.96	0.005	0.100
		M	0.88	0.005	0.059
1993-D	0.98	L	**0.98**	**0.008**	**0.222**
		G	0.95	0.008	0.130
		M	0.83	0.011	0.086
1994-A	1.0	L	**0.97**	**0.026**	**0.409**
		G	0.92	0.029	0.297
		M	0.84	0.032	0.198
1994-B	1.0	L	**0.96**	**0.031**	**0.393**
		G	0.90	0.034	0.268
		M	0.83	0.034	0.203
1994-C	1.0	L	**0.94**	**0.034**	**0.362**
		G	0.94	0.025	0.268
		M	0.92	0.024	0.213
1994-D	1.0	L	**0.97**	**0.020**	**0.320**
		G	0.95	0.016	0.218
		M	0.90	0.018	0.166

[1] Y_f is the final proportion of disease incidence per plot. WMV-2 in 1993; mostly ZYMV and WMV-2 in 1994.

[2] The models used were the linearized forms of the logistic (L) [$\ln (y/(1 - y)) = \ln(y_o/(1 - y_o) + r_c t)$], Gompertz (G) [$-\ln(-\ln y) = -\ln(-\ln y_o) + r_d t$], and monomolecular (M) [$\ln (1/(1 - y)) = \ln(1/(1-y_o)) + r_M t$].

[3] Standard error of the slope parameter.

[4] Average apparent infection rates estimated as the slope of a simple linear regression of disease incidence values transformed appropriately for the logistic, Gompertz, or monomolecular model.

Table 3.4. Paired *t*-test comparison of average apparent infection rates estimated with the linearized logistic model fitted to potyvirus epidemics in watermelon grown during the spring season of 1993 and 1994.

Year /	Rate	Std.	Estimated t_{ex} -values for[3]:						
Plot	(k_i)[1]	Error[2]	1993-B	1993-C	1993-D	1994-A	1994-B	1994-C	1994-D
1993-A	0.225	0.009	0.0001	2.25 *	0.24	6.47**	5.17**	3.78**	4.27**
1993-B	0.225	0.017		1.58	0.16	5.77**	4.73**	3.54**	3.58**
1993-C	0.193	0.011			2.09 *	7.43**	6.06**	4.65**	5.52**
1993-D	0.222	0.008				6.62**	5.30**	3.93**	4.46**
1994-A	0.409	0.026					0.39	1.07	2.65 *
1994-B	0.393	0.031						0.67	1.97
1994-C	0.362	0.034							1.04

[1] Average apparent infection rate of the logistic model estimated with the slope of simple linear regression of linearized data.

[2] Standard error of estimated epidemic rate parameters (k_i).

[3] Rejection of the hypothesis Ho: k_{ij}-k_{ij} = 0, with P = 0.05 (*) or P = 0.01 (**). The k_{ij} and k_{ij} are epidemic rates associated to epidemics *i* and *j*, respectively. Values are absolute estimates of *t* with the equation: $t_{ex} = k_{ij}-k_{ij} / [$ (Std. error (k_{ij})+Std. error (k_{ij})]".

Yield Loss Modeling. Average yield of watermelon fruits by single plant or by all plants infected at the same time were well correlated with the period in days in which plants remained healthy (Table 3.5).

The highest r^2-value was achieved with a simple linear model without intercept. No quadratic model was found with appropiate goodness-of-fit. Yield increase by plant was directly proportional to the period in which plants remained healthy with a rate of 0.146 kg day^{-1} (1993) and 0.140 kg day^{-1} (1994). In 1993, average fruit sugar content of plants that remained healthy the same period of time were not correlated with time of infection (r^2 = 0.046, P > F = 0.50). Significant correlation of sugar content with time of infection was found, however, in 1994 (r^2 = 0.818 P > F = 0.05).

Table 3.5. Yield loss and fruit sugar detrimental models associated to field potyvirus epidemics in spring watermelon, Leesburg, FL.

Year	Model	bo	b1	r^2	Pr > F
	Yield loss models [1]				
1993	$Y = 0.146\ (t)$	-	s	0.97	0.0001
	$Y = 33.460 + 0.17\ (t)$	s	s	0.48	0.0500
1994	$Y = 0.140\ (t)$	-	s	0.96	0.0001
	$Y = -25.200 + 0.72\ (t)$	s	s	0.83	0.0015
	Reduction of fruit sugar models [2]				
1993	$Y = 19.840 + 0.013\ (t)$	ns	ns	0.05	0.5000
1994	$Y = -18.670 + 0.670\ (t)$	s	s	0.82	0.0500

[1] Y = Kilogram per fruit per plant, t = period in days that a particular plant remained healthy.
[2] Y = Solubles solids measured as degree Brix; t defined as before.

Disease Gradients. Disease gradients were best described with the nonstationary wave model based on correlation of observed vs. predicted values, examination of residual plots, and capability to fit a wider range of shapes of disease gradients. The model converged with all data sets analyzed although sums of squares of residuals (SSE) and plots of residuals indicated poor fit to the data as time increased (Table 3.6).

Although the exponential model had a better goodness of fit than the power law, either model proved less satisfactory when disease gradients had a clear wave shape (Table 3.7, Fig. 3.2-3.3). This was particularly evident with gradients of 2 May for plot A and 2 and 5 May for plot B. The r-square values for these data sets were in the range of 0.90-0.98 vs. 0.84-0.76 for the nonstationary wave model and the best fitted empirical model (the exponential), respectively.

When a negative-exponential gradient type was observed (i.e. 8 May, plot B), a slight improvement was observed with the exponential model with respect to the nonstationary wave model (r^2 = 0.94 vs. r^2 = 0.92, respectively) (Table 3.7, Figure 3.3).

Table 3.6. Diffusion and curve shape parameters of disease gradient from a viral source into two watermelon plots estimated with the nonstationary wave model. Spring 1994.

Date	Plot	Inc[1]	Parameter estimated[2]	C.I. 95%[3] Lower	Upper	SSE[4]	r-square[5]	Pr > F[6]
2 May	A	8.0	m = 1.015	- 1.2253	3.2570	12.7	0.90	0.0039
			n_o = 6.204	4.2332	8.1759			
			D_o = 0.012	0.0017	0.0234			
	B	7.7	m = 3.340	- 0.1423	6.8231	2.5	0.96	0.0007
			n_o = 6.306	5.0099	7.6039			
			D_o = 0.011	0.0041	0.0189			
5 May	A	13.7	m = 0.956	- 3.4270	5.3396	224.6	0.65	0.0513
			n_o = 11.648	2.8028	20.4938			
			D_o = 0.012	- 0.0128	0.0380			
	B	12.0	m = 1.670	0.5498	2.7914	4.7	0.98	0.0002
			n_o = 9.524	8.5577	10.4919			
			D_o = 0.018	0.0125	0.0236			
8 May	A	20.8	m = 0.951	- 3.1420	5.0449	355.3	0.71	0.0357
			n_o = 16.388	5.7320	27.0457			
			D_o = 0.010	-0.0076	0.0284			
	B	16.7	m = 0.666	- 2.3151	3.6484	26.2	0.92	0.0026
			n_o = 8.820	3.6682	13.9729			
			D_o = 0.004	- 0.002	0.0112			
12 May	A	85.9	-	-	-	-	-	-
			-	-	-			
			-	-	-			
	B	82.9	m = 0.141	- 0.9740	1.2574	508.6	0.55	0.0898
			n_o = 14.018	3.5192	24.5180			
			D_o = 0.081	- 0.0288	0.1924			

[1] Total accumulated incidence of diseased plants per plot given as a percentage.

[2] Parameters estimated with the one dimensional nonstationary wave model. m = shape parameter of the gradient, n_o = parameter of reference of population density of the aphid vectors, D_o = is a diffusion parameter.

[3] Asymptotic 95% confidence interval per parameter estimated.

[4] Sum of squares of error associated with the model fitted to the data.

[5] Goodness of fit of simple linear regression of actual vs. predicted values estimated with the nonstationary wave model.

[6] Significance of the percent of variance explained by the simple linear regression model.

Disease gradients, as described with the nonstationary wave model are as follows: the predicted and actual data were well correlated on 2 May for plot A and of 2, and, 5 and 8 May for plot B ($r^2 \geq 0.90$, $Pr < 0.004$). Data from 5 and 8 May for plot A were fairly well fit by the model ($r^2 \geq 0.65$, $Pr < 0.052$). The data of 12 May was poorly described for plot B ($r^2 = 0.55$, $Pr < 0.089$) (Table 3.6).

The tendencies excluding results of the last date for plot B are: the diffusion parameter (D_0) in general decreased with time from 0.012 to 0.010 for plot A and from 0.011 to 0.004 for plot B. Statistically, only $D_0 = 0.018$ (5 May, plot B) and $D_0 = 0.004$ (8 May, plot B) were significantly different, as indicated by a non-overlapping confidence interval at P=0.05 (C.I. 95%) (Table 3.6). The shape of the gradient (m) also decreased with time from values higher than 1 to values which were close to or lower than 1, although no statistical differences were detected.

Wave shapes were observed in the initial disease gradient analyzed (2 May) and shifted to the negative exponential gradient six days later (Figs. 3.2, 3.3). The population reference parameter (n_0) increased with time as expected since the accumulative number of infected plants in the source increased. Statistically, only $n_0 = 6.306$ (2 May, plot B) and $n_0 = 9.524$ (8 May, plot B) were significantly different (P=0.05) (Table 3.6).

The diffusion parameter and the n_0 were similar on the first date for both plots ($D_0 = 0.012$ and 0.011, $n_0 = 6.2$ and 6.3), but the shape parameter on the first date differed ($m = 1.02$ and 3.34). Differences were higher in the following dates although comparisons may not be appropriate because of strong discrepancies in model goodness-of-fit on those dates. In any case, statistical differences were not detected in parameters values between plots as indicated by the overlapping of confidence intervals at P=0.05 (C.I. 95%) (Table 3.6).

Table 3.7. Parameters estimated with the linearized form of the power law and exponential models to describe disease gradients from a viral source into two watermelon plots.

		Power Law Model [1]				Exponential Model [2]			
Date	Plot	ln (a)	b	r^2	Pr > F [3]	Ln (a)	b	r^2	Pr > F
2 May	A	17.52	3.809	0.80	0.016	5.89	0.065	0.84	0.010
	B	11.38	2.303	0.61	0.067	4.52	0.042	0.67	0.047
5 May	A	20.05	4.318	0.66	0.050	6.84	0.073	0.68	0.043
	B	15.72	3.290	0.70	0.038	5.90	0.060	0.76	0.024
8 May	A	19.14	3.987	0.71	0.035	6.96	0.067	0.74	0.028
	B	12.63	2.446	0.91	0.003	5.28	0.043	0.94	0.001
12 May	A	-	-	-	-	-	-	-	-
	B	6.61	0.546	0.60	0.070	4.94	0.009	0.57	0.084

[1] Parameters estimated with the power low model (Gregory's model): ln (y) = ln (a) - b ln (x); y = estimated disease incidence at distance xi from the source. Distance in meters.
[2] Parameters estimated with the exponential model: ln (y) = ln (a) - bx; y and x as before.
[3] Significance associated to the coefficient of determination (r^2).

Serological tests. On 7 June 1993, WMV-2 was detected with ELISA in 286 out of the 294 plants of plot C. WMV-2 was also detected with the SDS-immunodiffusion test in the 8 samples obtained from all four plots on 28 June. PRSV-W and ZYMV were not detected in either test (Table 3.8). In 1994, seven plants were positive for ZYMV, and one reacted to antiserum to WMV-2 in SDS-immunodiffusion assays on 28 April (Plate 3.1). A total of 212 plants were positive for ZYMV, 9 for WMV-2, and 26 for both viruses in ELISA tests from samples taken on 26 May, 1994 (Table 3.8). Three plants from plot A that were positive for ZYMV in SDS-immunodiffusion tests were negative with ELISA when again sampled at the end of the season. Two of these three plants showed some mottling and leaf deformation characterized by size reduction and strong basal constrictions in each lobed sector. The remaining plant showed some vein-clearing, vein-banding and light rugosity. Of the three plants, this was the only one positive for WMV-2 in ELISA. WMV-2, but not ZYMV, was also detected in four commercial fields sampled on 18 and 21 May.

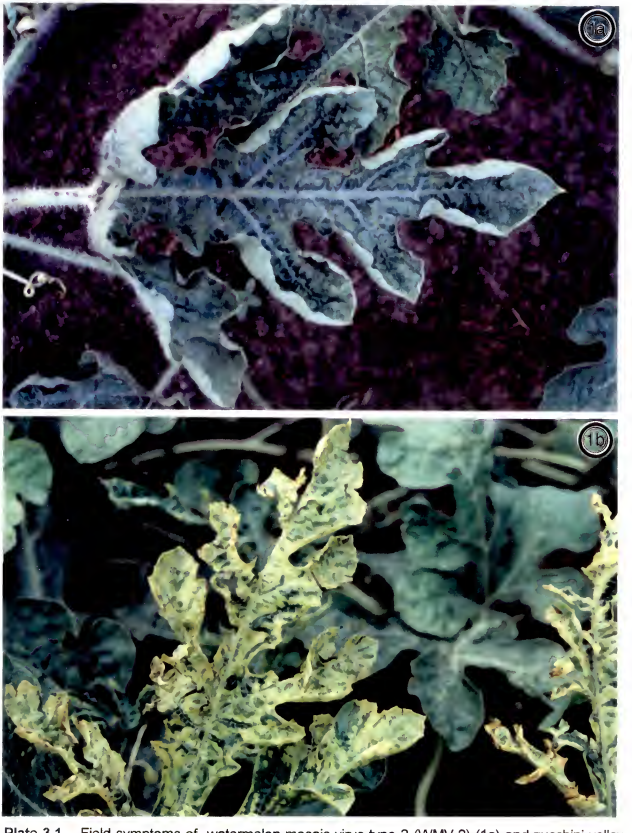


Plate 3.1. Field symptoms of watermelon mosaic virus type 2 (WMV-2) (1a) and zucchini yellow mosaic virus (ZYMV) (1b) in watermelon. Virus infections were verified with SDS-immunodiffusion tests. Isolates were coded FC-3328 (WMV-2) and FC-3326 (ZYMV). Spring 1994, Leesburg, FL.

Table 3.8. Result of ELISA and SDS-immunodiffusion tests for viruses in watermelon (*Citrullus lanatus*) in north Central Florida in 1993-1994.

Year/ Plot[1]	Sampl. Date	No. of Assays[2]	ELISA : Samples positives for[3]:					
			WMV-2	PRSV-W	ZYMV	WMV-2 PRSV-W	ZYMV PRSV-W	WMV-2 ZYMV
93/C	7June	294	286	0	0	0	0	0
94/A	26 May	298	9	0	212	0	0	26

			SDS-Immunodiffusion : Samples positives for:						
			WMV-2	PRSV-W	ZYMV	SMV	CMV	TV	2932
93/A-D	28 June	8	8	0	0	0	0	0	0
94/A,B,C	2 May	8	1	0	7	0	0	0	0
94/ fields	18 May	4	4	0	0	0	0	0	0

[1] Year and plot sampled.

[2] Number of samples assayed per year and plot. Total plot sampling was done for ELISA tests on the dates indicated. For ELISA-1994 only 80 plants were tested for PRSV-W; rows 1, 5, 12 and 15 of plot A were systematically sampled. Sampling based on symptoms (1993 season) and total sampling of infected plants (1994 season) were done on the dates indicated for SDS-immunodiffusion.

[3] Number of samples that were positive based on their reactivity to antisera to watermelon mosaic virus type 2 (WMV-2), papaya ringspot virus type w (PRSV-W), zucchini yellow mosaic virus (ZYMV), cucumber mosaic virus (CMV), squash mosaic virus (SMV), trichosanthes virus (TV), and an uncharacterized potyvirus (coded as 2932). WMV-2+PRSV-W, ZYMV+PRSV-W, and WMV-2+ZYMV indicate mixed infections of the viruses indicated.

Discussion

Potyvirus epidemics in cucurbits can be characterized for seasonal and regional effects on the intensity of epidemics, for seasonal variability in composition and prevalence of viruses infecting a the crop, and for the importance of abundance and closeness of infected host(s) for primary viral infection to occur (Hander et al., 1993; Webb and Linda, 1993; Castle, 1992; Dahal, 1992; Lecoq et al., 1991b; and Wang et al., 1991; Perring et al., 1989; Gray et al., 1986; Adlerz, 1974a; 1978a; Nelson and Tuttle, 1969).

In this framework, the results of this research agree with previous findings that the most common viral disease affecting spring watermelon production in North Central Florida is WMV-2 (Webb and Linda, 1993; Purcifull et al., 1988; Adlerz et al., 1984).

However, under specific conditions, ZYMV may induce epidemics with higher epidemic rates, and thus could induce greater yield losses than WMV-2. Evidence is also provided that nearby viral sources may play an important role in providing primary inoculum, but significant secondary dispersion may also occur within fields.

Although ZYMV was first found in the United States in 1981 in squash in Florida (Adlerz et al., 1983; Purcifull et al., 1984a), erratic appearance of this virus has been reported throughout the state (Webb and Linda, 1993; Purcifull et al., 1988; Adlerz et al., 1983) and no high epidemic intensities in watermelon or any other cucurbit crop due to ZYMV alone or in mixed infections with other viruses has been documented in Florida.

This study shows that ZYMV was able to spread faster than WMV-2 in the 1994 spring season, but ZYMV was not detected in the spring of 1993. Because the final disease incidence caused by WMV-2 alone or mixed with ZYMV was lower than 12%, it can be stated that ZYMV alone was mostly responsible for the epidemic characteristics in the experimental plots in 1994.

Characteristics of 1994 epidemics included higher epidemic rates, shorter epidemic duration, earlier onset of epidemic and shorter time to reach 50% incidence than the epidemics of the 1993 season. In addition, a fruit yield loss of 31%, in comparison to a 26% in 1993, were estimated in 1994. Significant reduction of sugar content below 11 °Brix per fruit, the standard for the cultivar, was only recorded in this year.

Because other factors such as weather, vector activity, and proximity of inoculum sources may influence the development of epidemics, it can not be concluded definitively that the epidemiological differences between the two seasons are due to the predominant virus. However, our results agreed with findings in France where ZYMV appears to be endemic in squash since 1979 (Lecoq et al., 1981).

Lecoq and coworkers showed faster spread of ZYMV epidemics in squash than those induced by WMV-2 even though both types of epidemics reached 100% incidence (Lecoq et al., 1991b). Similarly, competitiveness of ZYMV over WMV-2 and PRSV-W in mixed infections both in fall squash crops and under experimental conditions was also reported in New Jersey (Davis and Mizuki, 1987).

However, in southern California, where WMV-2 has been reported at least since 1965 (Webb et al., 1965) as opposed to the relatively recent detection of ZYMV in 1983 (Provvidenti et al., 1984), contrasting results have been noted (Castle, 1992). In Castle's studies, although both types of epidemics reached 100% incidence, epidemics of WMV-2 in muskmelon spread faster than those induced by ZYMV in at least three of five epidemics (Castle, 1992). Castle (1992) attributed the faster spread of WMV-2 to the existence of more alternative hosts due to longer endemicity of this virus than ZYMV. Elsewhere in the United States, WMV-2, PRSV-W or both, but not commonly ZYMV, have frequently been found inducing high epidemic intensities in cucurbit crops (Hander et al., 1993; Gray et al., 1986).

Although epidemiological work has to be continued in Central Florida to determine further prevalence of ZYMV after its high incidence in 1994, results of 1993 support previous indications of low and erratic incidence of this virus, including no detection in spring 1989 (Webb and Linda, 1993). Although this study was not designed to explain the low incidence of ZYMV, several explanations can be given based on the plant-vector-virus system.

Regarding the vector component, with the exception of *Lipaphis erysimi* and *Brachycaudus rumexicolons*, the known vectors of ZYMV also transmit WMV-2, the most widespread virus in Central Florida (Table 2.1, Table 2.4). In addition, some vectors such *Myzus persicae*, *Aphis gossypii*, *A. spiraecola*, *A. middletonii*, *Acyrthosiphon kondoi* and *A. pisum* transmit ZYMV at higher rates than those with WMV-2 (Castle et al., 1992; Adlerz, 1987). Because of this, it can be argued that

composition and perhaps numerical density of aphid vectors alone does not explain the lack of detection of ZYMV in 1993 in this work and in previous epidemiological studies (Webb and Linda, 1993). This argument will be extended in Chapter 4 when dealing with correlative studies of aphid vectors and disease incidence.

On the other hand, analysis of disease gradients from plots A and B in 1994 (Table 3.6), although not extensive, clearly suggests that closeness and numerical density of infected hosts, in this case another infected watermelon crop, may play an important role in the prevalence of ZYMV. The predominance of ZYMV in 1994 with respect to previous years, suggests that it was due to specific local conditions. The field from which the virus may have arrived apparently was accidentally transplanted with infected plants (D. L. Hopkins, personal communication). However, the virus in the transplants was not identified prior placing them in the field.

The presence of local sources has been used to explain the occurrence of epidemics of WMV-2 (Gray et al., 1986; Nelson and Tuttle, 1969) and PRSV-W (Hander et al., 1993) elsewhere. In South Florida, where PRSV-W is endemic and has become the most limiting virus disease in cucumber production (Adlerz et al., 1983), it has been shown that the wild cucurbits *Momordica charantia* L. and *Melothria pendula* L. provide, at least locally, the primary inoculum for epidemics to occur (Adlerz, 1974b, 1972a, 1972b).

Previous attempts to demonstrate the effect of local sources on the spread of viral diseases in watermelon and other cucurbits were based on empirical observations (Hander et al., 1993; Castle, 1992; Gray et al., 1986; Nelson and Tuttle, 1969; Adlerz, 1974b, 1972a). In this study, modeling of disease gradients was used as epidemiological proof of the effect of a local source in the development of epidemics. The model selected provided, based on ecological principles, biological information not available from other commonly used models in plant disease epidemiology (Campbell and Madden, 1990).

The nonstationary wave model is characterized by a lack of asymptotic behavior (thus a finite distance is considered), and describes a dispersion "wave" type which is not constant through time. These characteristics provide a flexibility that is not present in other types of models based on the classical diffusion equation (Minogue, 1989). Although this model and similar ones based on the classical diffusion model were suggested to describe diffusion of animal and insect populations (Rudd and Gandour, 1986; Shigesada, 1980; Okubo, 1986, 1980), its application to the ZYMV / WMV-2 - watermelon system seems acceptable because aphids are involved as dispersion agents. The basic assumption used to biologically interpret the model was that the number of infected plants are estimators of the number of successful events of acquisition-transmission by vector species.

In addition to demonstrating viral dispersion from a local source, the nonstationary wave model provided a speed and shape parameter important to understanding biological attributes of the system under study. For example, poor fit to the model in the last two dates for each plot suggests that, in addition to a local sources, there are other factors that contribute to the build-up of viral epidemics in watermelon.

Secondary spread within the field appears to occur based upon spatial data and the shape parameter values ($m > 1$) obtained in this data. The wave shape ($m>1$) suggests a border effect that gradually is lost as the epidemic progresses ($m< 1$) to an almost horizontal line to the x-axis which indicates absence of distance dependence. In addition, Q-values (interpreted as the numbers of aphid vectors that successfully acquire the virus in the source) used to fit the nonstationary wave model were smaller than some values of the estimated number of viruliferous aphids that successfully inoculated a virus. Thus, it can be suggested that arrival of vectors from different sources may also occur.

Although an initial distance of 40 m from the field source was used to determine disease gradients, it can be suggested that individual sources within the field in the range of 40-200 m (the closest and farthest distance from the plots A and B), could have contributed to the dispersal gradients found in this study. From a practical point of view, establishment of commercial watermelon fields at least 200 meters from each other may delay epidemic development of ZYMV, and perhaps WMV-2, under the conditions of Central Florida. This could especially be important if different planting dates are used by the neighboring growers. This management practice may also include an additional separation from field borders. Under conditions of South Florida, Adlerz (1974b; 1972a) indicated, based on empirical observations, that spread of PRSV-W into watermelon and other cucurbit fields was limited to 15-38 m from infected weeds (i.e., *Melothria pendula*) growing on field borders.

The other component in the plant-vector-virus system that could explain the erratic ocurrence of ZYMV is that the virus may become nontransmissible or poorly transmissible by aphids. Whereas WMV-2 has been shown to be more stable regarding transmissibility properties (Castle, 1992), great variability of ZYMV has been recognized when long maintained through mechanical passages (Huet et al., 1994; Granier et al., 1993; Gal-On et al., 1992; Lecoq and Purcifull, 1992; Lecoq et al., 1991a; Lecoq, 1986). Yet, the transmissibility of ZYMV may also be reduced due to the age of the infection (Castle, 1992), perhaps because of low virus titer in the plant.

The last aspect may support findings of this study regarding some serological assays conducted in 1994. Despite the difference in the serological method and the polyclonal antisera used in the assays, the negative ELISA-reaction in three plants that were previously positive for ZYMV with the SDS-immunodifusion test suggests a drop of virus titer about one month after the infection took place. Yet, mild symptoms were observed at the sampling time (26 May). Although the sampling method can not be ruled out as a way to explain these results, the directed (non-random) and multiple-leaf

sampling from symptomatic runners should have reduced bias to very low levels. The serological results appear to agree with field and greenhouse observations of apparent recuperation of plants through a transitional process of strong yellowing, distortion of runners, moderate to severe necrosis, and finally production of new asymptomatic or lightly mottled tissue, often from lateral-basal runners. Such pathological behaviour has been observed by H. Lecoq (D. E. Purcifull, personal communication) in cantaloupe infected with ZYMV.

It can be hypothesized that the genomic variability of ZYMV could eventually result in changes in structural and nonstructural proteins directly involved in vector transmission (i.e. coat protein and helper component), thus affecting aphid transmissibility during the season. Lecoq et al. (1991b) showed that mild strains obtained from asymptomatic squash tissue still were vector-transmissible. Because the study of Lecoq et al. (1991b) was not comparative, the idea of production of isolates with defective structural and nonstructural proteins during the growing season can not be ruled out. Even if defective helper component is not produced, the proportion of successful events of virus acquisition should be reduced due to a restricted period of adequate virus titer in the plant. Castle (1992), observed reduction of transmissibility of ZYMV by *M. persicae* whereas the virus titer declined under greenhouse conditions. Presence of severe and mild strains of ZYMV has already been demonstrated in Florida (Wisler et al., 1995), but variability of ZYMV during the growing season and its etiological and epidemiological consequences, such as diagnosis, and prevalence, still need to be fully studied. Overall, perhaps the plant-virus-vector system as a whole has to be addressed from the evolutionary perspective of a relatively new component in the Florida agroecosystem in process of adaptation.

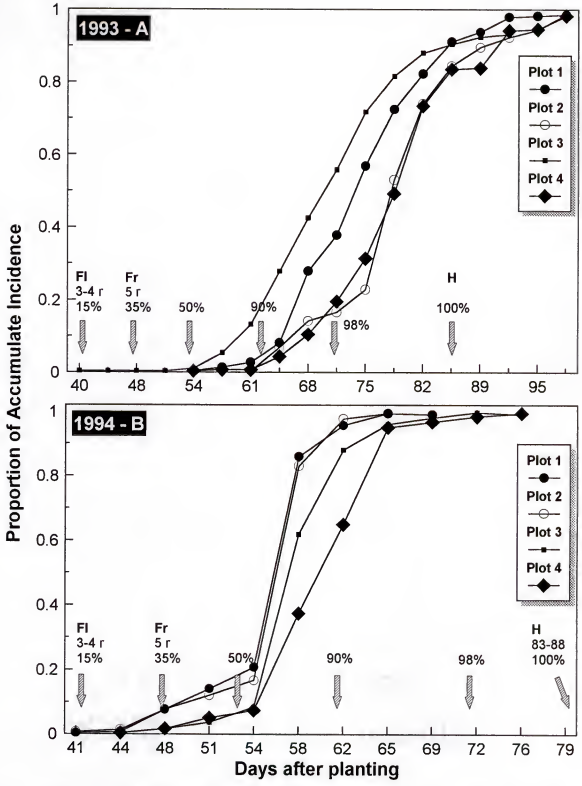


Figure 3.1. Disease incidence due to watermelon mosaic virus (WMV-2) in 1993 **(A)** and Zucchini yellow mosaic virus (ZYMV) and WMV-2 in 1994 **(B)** in watermelon. Arrows indicate time in days after transplanting when flowering (Fl), fruit setting (Fr), and harvest (H) occurred in watermelon cv. 'Fiesta'. Percentages indicate ground covered with watermelon foliage.

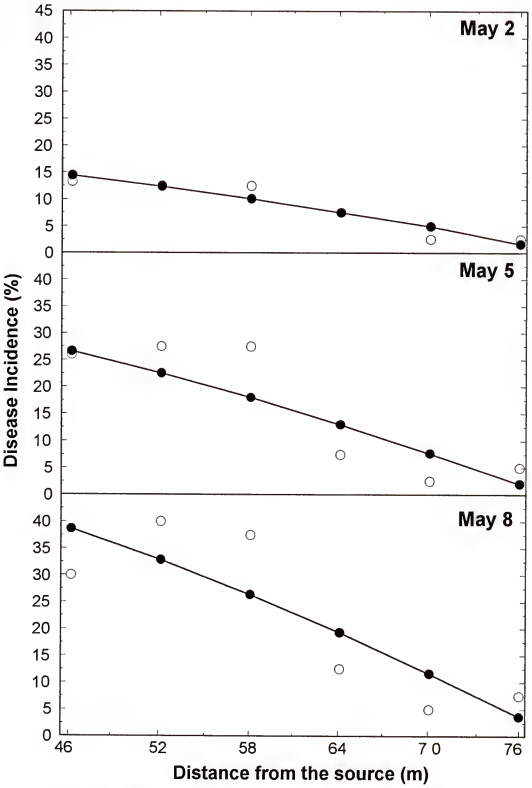


Figure 3.2. Gradient dispersion of disease incidence due to watermelon mosaic virus (WMV-2) and zucchini yellow mosaic virus (ZYMV) from a field of infected watermelon to a watermelon plot in 1994 (○) and fitted gradient with the nonstationary wave dispersion model (—●—) on 2 May, 5 May, and 8 May 1994, in plot A.

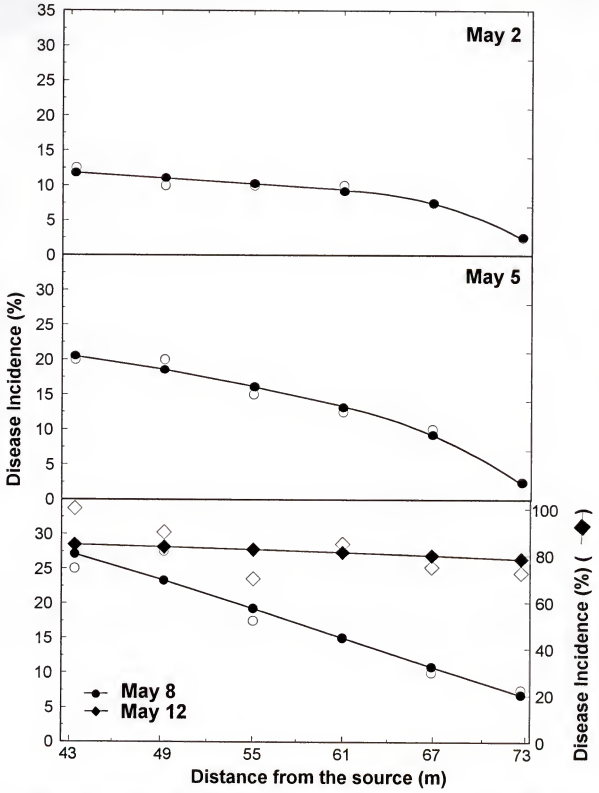


Figure 3.3. Gradient dispersion of disease incidence due to watermelon mosaic virus (WMV-2) and zucchini yellow mosaic virus (ZYMV) from a field of infected watermelon to a watermelon plot in 1994 (○, ◆) and fitted gradient with the nonstationary wave dispersion model (—●—) on 2 May, 5 May, 8 May, and 12 May (—◆—) 1994, plot B.

CHAPTER 4
STATISTICAL CORRELATION OF APHID VECTOR SPECIES WITH VIRAL DISEASE INCIDENCE IN WATERMELON

Introduction

Transitory, noncolonizing aphids as well as colonizing aphid species play an important role in the development of most virus epidemics. These aphid species may arrive from long distance or local sources carrying the primary or secondary inoculum to build up epidemics, providing that they had probed on infected hosts and that they find a population of healthy, susceptible plants (Taylor, 1986; Raccah, 1986; Taylor, 1984). Understanding the vector component as it relates to the development of epidemics is to a great extent the cornerstone of plant virus epidemiology. This component has proven to be rather complex due to the abundance and diversity of species, variable interaction with the plant-virus system, and behavioral factors (Webb, et al., 1994; Huet et al., 1994; Granier et al., 1993; Gal-On et al., 1992; Webb, 1992; Lecoq et al., 1991a; Lecoq, 1986; Irwin and Kampmeier, 1989; Irwin and Ruesink, 1986). Yet, knowledge of the vector component in the epidemiological context is fundamental in plant disease management.

Aphid vectors in Central Florida

Several studies have been carried out to determine the species composition of alate aphids present in cucurbit production areas and to determine the transmissibility of WMV-2, ZYMV, and PRSV-W by their aphid vectors in North Central Florida. An extensive study was conducted with naturally infectious alates caught in screen, suction, and yellow water pan traps during spring epidemics in cucurbit plantings. In

three years, only 4 out of 40 species transmitted WMV-2, ZYMV, or both, but none transmitted PRSV-W (Adlerz, 1987). Of these, 92% of all viruliferous aphids were either *Aphis spiraecola* (52%) or *A. middletonii* (40%). Both of these aphids transmitted WMV-2 and ZYMV. The remaining 8% of viruliferous vectors were *Myzus persicae*, which transmitted ZYMV and *A. illinoisensis,* which transmitted WMV-2. The first viruliferous aphid trapped by Adlerz (Adlerz, 1987) was *A. middletonii*, confirming results from an earlier study (Adlerz, 1978b) in which this species was found to be the only vector trapped on most days during a severe epidemic outbreak. This aphid transmitted WMV-2 every time a test was conducted with naturally infectious alates trapped in fields with a disease incidence of at least 4%. Adlerz (1987) concluded that only two or three aphid species (i.e., *A. spiraecola*, *A. middletonii*, and possibly *M. persicae*) were important virus vectors in spring epidemics in North Central Florida.

Recently, Webb and Kok-Yokomi (1993) first reported *Uroleucon pseudambrosiae* as a vector of WMV-2 and PRSV-W, suggesting that this aphid could play an important role in spring epidemics of these viruses in watermelon in Florida. This aphid, which was caught in large numbers in green tile water pan traps (Webb et al., 1994), was able to transmit WMV-2 (isolate G2301) in arena tests (23% ± 12% SD, N=15) and controlled tests (53 out of 450 plants tested). PRSV-W was also successfully transmitted in arena tests (41% ± 22 SD, N=10). An aphid-transmissible (*M. persicae*) Florida isolate of ZYMV was not transmitted by *U. pseudambrosiae* in preliminary experiments, either in arena tests or in controlled access tests.

Stochastic models

Most modeling studies have been carried out to find the association of vector activity with changes of viral disease incidence (Mora-Aguilera et al., 1993a; 1992; Madden et al., 1990; Madden et al., 1987c; Clement et al., 1986; Marcus and Raccah,

1986; Sigvald, 1986; Ruesink and Irwin,1986; Watson and Healy, 1953). Although there are some reports where relationships between vector dynamics and progress of virus epidemics have not been found (Basky, 1986; Toba et al., 1977), such relationships are generally well documented (Madden et al., 1990; Clement et al., 1986; Sigvald, 1986). In finding the association of aphid vectors and changes in the incidence of the diseases that they transmit, two general approaches have been used: the stochastic and the deterministic approach. In contrast to the deterministic approach, stochastic models estimate the average vector propensity (*sensu* Irwin and Ruesink, 1986) through statistical models rather than through experimental means. Yet, stochastic models have been proven to be adequate for general descriptions of epidemics (Mora-Aguilera et al., 1993a; Plumb et al., 1986; Raccah et al., 1988; Madden et al., 1983).

Regression analysis, particularly multiple regression models, has been used for modeling changes of viral disease incidence, as a dependent variable, in relation to vector species, each species being considered an independent variable. Positive associations between these two components have been found in studies in which lag time adjustments were considered. These lag time adjustments can be explained biologically as the average incubation time required for viral symptom expression after inoculation. The final objective in most studies of this type is to define the most important vector species (Mora-Aguilera et al., 1993a; Plumb et al., 1986; Raccah et al., 1988; Madden et al., 1983), rather than support a specific control program as the classical idea of forecasting suggests (Madden and Campbell, 1990; Madden and Ellis, 1988; Chuang and Jeger, 1987; Coakley et al., 1985). When the intent of regression studies with lagged variables departs from the classical idea of forecasting, then this term should perhaps be avoided. The term "correlation" could be used instead.

In a study with epidemics of papaya ringspot virus type-P in *Carica papaya,* a series of regression models were developed and validated with 60 PRSV-P epidemics

in a five year study (Mora-Aguilera et al., 1993a). In this study, lag-counts of *A. nerii (An)*, *A. gossypii (Ag)* and the interaction of speed and wind duration (*W*) with precipitation (*P*), predicted changes of disease incidence in 40% ($R^2 \geq 60\%$) of the epidemics (24 out of 60). The forecasting model selected after a validation process was $\hat{y} = 0.442 + 0.168\ An + 0.658\ Ag + 0.000092\ PW$.

Madden et al. (1983), and Raccah et al. (1988), used regression models to identify important aphid species in the spread of maize dwarf mosaic virus (MDMV) in *Zea* sp, and tobacco etch (TEV) and tobacco vein mottling (TVMV) viruses in *Nicotiana tabacum*. Even though regression models were applied successfully in these studies, caution should be taken in applying this method when colonization of plants by aphid vectors may take place, and/or when viruses are persistently transmitted. Aphid vectors of MDMV, TEV, and TVMV are able to colonize maize and tobacco (Madden 1993, personal communication), which means that it is not possible to separate specimens that have immigrated into the field before being caught in traps from those that are moving within the field. Additionally, the possibility of colonization (Madden et al., 1983; Raccah et al., 1988) implies secondary virus dispersion by apterous aphids which is not considered in the regression models.

Another precaution that should be taken in the application of regression models concerns the problem of multicollinearity. Multiple regression analysis is often affected by multicollinearity, i.e., the presence of variable(s) highly correlated with other variable(s) in the data set, thus reducing the robustness or reliability of inferences (Jolliffe, 1986; Hawkins and Fatti, 1984). Although multicollinearity can be detected within multiple regression models by computing specific statistics such as VIF (variance inflation factor), it can not be corrected (Mora-Aguilera et al., 1993a; Chuang and Jeger, 1987). Therefore, approaches to identify, correct, or reduce multicollinearity are fundamental to forecasting plant virus epidemics with stochastic models. There are several approaches, including two multivariate techniques, principal component

analysis (PCA) and biplot displays, to reduce and eventually eliminate multicollinearity and spurious variables, a process referred to as reduction of dimensionality (Jolliffe, 1986; Dunteman, 1989).

Two additional drawbacks of the multiple regression analysis are that, the method may fail to provide specific biological explanations regarding interactions in the host-virus-vector(s) system, and that important variables could be eliminated. Reliability of the method regarding these two problems is determined by the model structure (Draper and Smith, 1981; Freud and Littell, 1985).

In summary, it is possible to use multiple regression models to study the relationship between a nonpersistently transmitted virus disease and its vectors in the absence of colonization. WMV-2, PRSV-W, ZYMV, PRSV-P, and other viruses share these characteristics (Webb and Kok-Yokomi, 1993; Adlerz, 1987; 1978b; 1974a; Halbert et al., 1981; Van Harten, 1983; Mora-Aguilera et al., 1992). Regression models, applied correctly, could be used when a general description of the structure of an epidemic is required.

It was demonstrated in Chapter 3 that epidemics in watermelon were mostly caused by WMV-2 and ZYMV, during 1993 and 1994, respectively; that the average apparent infection rates were significantly higher in 1994; and that local sources of virus may play an important role in the progress of epidemics. The objective of this study was to identify the most important aphid vectors associated with the contrasting viral epidemics caused by WMV-2 in 1993 and by ZYMV in 1994 in spring watermelon in Central Florida. This objective was approached by combining principal component analysis and varimax rotated biplots with regression analysis of counts of aphid vectors as independent variables with changes of disease incidence of ZYMV and / or WMV-2, as the dependent variable. By selecting a reduced number of aphid vectors, more refined ecological studies could be conducted to allow a better understanding of the

development of viral epidemics in watermelon in Central Florida. Thus, eventually, specific clues could be provided for disease management.

Materials and Methods

Establishment of field plantings. In 1993 and 1994 four experimental plots were established in a 6-ha field at the Central Florida Research and Education Center at Leesburg, FL. Each plot consisted of 300 plants planted in 15 east-west oriented rows 30 m long, spaced 3 m apart with plants separated by 1.5 m within rows. Plots were surrounded by 4 m of bare soil and separated by 60 m of bahia grass pasture east-west and 30 m north-south from each other. On 24 March (1993) and 14 March (1994), the watermelon cultivar 'Fiesta' was directly seeded in raised beds at a rate of 3-4 seeds per hill. To reduce wind damage and sand-blasting of young seedlings, windbreaks of rye were established between every other row in late January and removed on 21 April in both years. In 1993 several experimental fields of cucurbits were located to the west and southwest of the watermelon plots. A vineyard (*Vitis* spp.) was located east of the plots. In 1994, an experimental field of about 1.5 ha, used in watermelon breeding trials, was located south of plots A and B.

Additional information regarding row thinning, schedule of insecticide and fertilizer applications, windbreaks to reduce wind damage, and other cultural practices was provided in the Materials and Methods section of Chapter 3.

Disease assessment. Each of the 300 plants per plot was observed once every 3 days from emergence on 29 March (1993) and 19 March (1994) to harvesting on 24 June (1993) and 2 June (1994) for symptoms typical of viral infection. Typical symptoms included distortion of leaves and ends of runners, and vein-banding, green spotting, mottle, vein-clearing chlorosis or moderate necrosis of the foliage. No attempt was made to associate these symptoms with any of the viruses previously found

infecting watermelon in Florida because of the great biological variability of these potyviruses and high possibility of mixed infections (Webb and Linda, 1993; Lecoq and Purcifull, 1992; Adlerz et al., 1983). The location of each individual diseased plant was recorded for each plot and evaluation date. Progress of incidence in plots was treated as four separate epidemics.

Serological tests. Enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977), was used with intensive sampling to determine the presence/absence of WMV-2, ZYMV, and PRSV-W, which are considered the most important and common viruses in watermelon in Florida (Webb and Linda, 1993; Purcifull et al., 1988; Purcifull et al., 1984a; Adlerz et al., 1983). Presence of these viruses and squash mosaic virus, cucumber mosaic virus, a posible potexvirus (Purcifull et al., 1988), and an unnamed potyvirus (coded as 2932) (Purcifull et al., 1991) previously found in cucurbits in Florida (Purcifull et al., 1988), were also determined with sodium dodecyl sulfate (SDS)-immunodiffusion (Purcifull et al., 1984a; Purcifull and Batchelor, 1977) in a small number of samples. Information regarding dates, type, and size of sampling for ELISA and SDS-immmunodiffusion tests, as well as specific antisera used was provided in Chapter 3.

Aphid monitoring. Alate aphids were captured in green tile water pan traps identical to those used in a previous experiment (Webb et al., 1994) from 16 April to 21 June in 1993, and from 21 March to 17 June in 1994. Traps were constructed by using a clamp to attach a clear plastic box (13 x 13.5 x 3 cm) to a 90 cm metal road. A dark green ceramic tile (10.8 cm x 10.8 cm) (ABC tiles, Orlando, FL 32805), which matched the color and spectral reflectance of watermelon leaves (Webb at al., 1994), was placed in the box. The pan trap was filled to 1/2 to 2/3 capacity with water containing a small amount of detergent to break the surface tension. A trap was placed in each of the two outside rows of each of the four corners of each plot (i.e., four pairs of traps). Traps were placed on the bed 30 to 60 cm from the second plant and oriented toward

the field. Trap height was initially set up 30 cm above the ground, then was adjusted up to 50-60 cm as the crop grew. Water was removed from each pair of traps daily Monday through Friday, placed in labeled plastic containers, and taken to the laboratory for sorting. During hot weather, when water evaporated rapidly, week-end collections were made on Saturday or Sunday.

Aphids from each pair of traps were counted and stored in 70% ethanol. A few specimens were identified in alcohol. *Uroleucon* - type specimens and other aphids that could not be identified in alcohol were cleared and mounted on microscope slides following the procedure outlined by Hille Ris Lambers (1950). Voucher specimens will be deposited with the Florida State Collection of Arthropods, Gainesville.

Selection of independent variables. Because only WMV-2 and ZYMV were found causing spring epidemics in 1993 and 1994 (Chapter 3), only potential vectors of these two viruses, as reported worldwide (Chapter 2: Tables 2.2-2.4), were initially considered as candidates for independent variables. Candidate species were further narrowed down to *Aphis gossypii, A. craccivora, A. spiraecola, A. middletonii, Myzus persicae,* and *Uroleucon pseudambrosiae,* based on their abundance and population dynamics and on reports in Florida and elsewhere of putative major vectors for WMV-2 and ZYMV (Webb and Kok-Yokomi, 1993; Castle et al., 1992; Yamamoto et al., 1986; Adlerz, 1987; 1978b; 1974a; Nelson and Tuttle, 1969).

Only the number of the alates of each aphid vector species was used as an estimator of viral incidence because, with the exception of *A. gossypii,* these aphids do not colonize watermelon plants (S. E. Webb, personal communication) Colonization of plants by *A. gossypii* was ignored, because this occurred at the end of the season, during the last phase of epidemics. The actual proportion of viruliferous aphids in vector populations was not determined.

Selection of dependent variables. Dependent variables were incremental changes in percent disease incidence averaged [$(Y_t - Y_{t-1}) / 4$], accumulated per the

four plots per year [Σ (Y_t - Y_{t-1})], and the arc sine of the square-root of this last variable { arc sine [Σ (Y_t - Y_{t-1})]$^{\frac{1}{2}}$}.

Model development and selection. Model development was comprised of two stages: 1) preselection of the independent variables, and 2) selection of a set of potential predictive models. Principal component analysis and varimax rotated biplots of the major principal components were integrated with multiple regression analysis to examine the correlation of the number of aphid vectors (as independent variables) with dependent variables. In stage one, the procedure PROC PRINCOMP of SAS was used to define the major (principal components with the highest variance, $\lambda \geq 1.0$) and minor principal components (those corresponding to the smallest variance, $\lambda << 1.0$) (Hawkins and Fatti, 1984). Variables with the highest loading or weight in a single eigenvector were selected to represent the respective principal component provided it had not been chosen to represent a principal component of the same category, i.e., major or minor. Variables associated with the minor principal component were eliminated because they either were correlated with other variables or were irrelevant in the data structure characterization.

To verify the preselection of variables, PROC FACTOR of SAS was used to obtain rotated major principal components and to produce displays of rotated biplots of these major principal components (PC). Varimax rotation was used to gain interpretability of both PC's and biplots, while still mantaining the orthogonality of the data (Jolliffe, 1989; 1986). Biplots are planar displays that allow inspection of the correlation among variables while helping to identify the variables that explain most of the variance in that particular display (Gabriel,1971). Long lines indicate variables that are close to the plane being displayed and are well represented with a large variance. Angles between the lines at (or near) 180° or at (or near) 0° indicate high negative or positive correlation between variables, respectively (Gabriel, 1971). Variables represented by the longest vectors and with lowest correlations were preselected.

In stage 2, the STEPWISE option of PROC REG of SAS was used to regress scores of principal components of the selected variables against the dependent variables. The linking of PROC FACTOR with PROC REG was done to use an orthogonal matrix of independent variables, thus avoiding dimensional problems and multicollinearity. Counts of the aphid species transformed as PC-scores were lagged with respect to the highest change of disease incidence. To narrow down the number of lagged variables being used to generate the final model(s), groups of lagged variables, which included scores for the different aphid species, were regressed with changes in disease incidence accumulated for the four plots per year [$\Sigma (Y_t - Y_{t-1})$]. The STEPWISE procedure of PROC REG of SAS Institute (1988) was also used at this stage. The variables selected from the different groups were pooled in combinations of 1-5 variables and regressed against the different types of dependent variables. The models were generated without an intercept because of its lack of biological meaning for this type of study. The choice of the best series of statistical models was based on the proportion of the variance accounted for by the model (R^2-values), significance of the R^2-values, significance of the regression parameter stimates ($\hat{\beta} i$), and Mallow's C_p statistic (Draper and Smith, 1981; Freud and Littell, 1985). All statistical analyses were done using SAS 6.08 for MS Windows™ (SAS™ Institute, Cary NC).

Results

Aphid abundance and species composition. A total of 37 aphid species were collected in the two seasons combined (Table 4.1). Twenty-seven species were trapped in 1993 and 32 in 1994; 22 of these species were collected in both 1993 and 1994.

Fifteen of the species trapped are known to be vectors of WMV-2, 8 are known vectors of ZYMV, and 11 species have been reported as vectors of PRSV-W (Table

4.1). Six of these species are known to be able to transmit all three viruses, five have been shown to transmit WMV-2 and PRSV-W, and two transmit both WMV-2 and ZYMV. *Rhopalosiphum maidis* (Fitch), *R. padi* (L.), and *Hysteroneura setariae* (Thomas) transmit WMV-2, *Brachycaudus helichrysi* (Kaltenbach) is known to transmit PRSV-W, and *Lipaphis erysimi* (Kaltenbach) transmits only ZYMV (Table 4.1). Because WMV-2 and ZYMV were found associated with epidemics in 1993 and 1994 (Chapter 3), Figure 4.1 shows the relative proportion of aphid species known to be vectors of these two viruses.

With the exception of *Tetraneura nigriabdominalis* (Sasaki), which has not been reported as a vector of any plant virus, the most abundant species of the 37 species trapped in the two seasons are known vectors of at least one virus: *Aphis craccivora* Koch, *A. gossypii* Glover, *A. spiraecola (=A. citricola)* Patch, and *Myzus persicae* (Sulzer) of WMV-2, ZYMV, PRSV-W; *Aphis middletonii* (Thomas) of WMV-2, ZYMV; *Uroleucon pseudambrosiae* (Olive) of WMV-2, PRSV-W, and *Lipaphis erysimi* (=*Hyadaphis pseudobrassicae*) (Kaltenbach) of ZYMV and PRSV. Photographs of cleared and mounted specimens, as well as photographs showing morphological identification features of these aphids are included in Appendix 1.

In 1993, *U. pseudambrosiae* (137 out of 462 total aphid vectors), *A. middletonii* (137 out of 462), and *A. gossypii* (119 out of 462) were the most common aphid vectors caught in green tile water traps. The highest peaks of these species were 14 May, 25 April and 5 May, respectively. *A. spiraecola*, *A. craccivora* and *M. persicae* were less frequently captured (Figures 4.5).

In 1994, the aphids *U. pseudambrosiae* (1390 out of 1803 total aphid vectors), *M. persicae* (113 out of 1803), and *A. middletonii* (97 out of 1803), were the most common aphid vectors caught. The highest peaks of these vectors were 15 April and 4 May (*U. pseudambrosiae)*, 25 April (*M. persicae*), and 20 April (*A. middletonii*) (Figure 4.6).

Table 4.1. Composition of aphid species and total individuals collected in green tile pan traps in watermelon plots at Leesburg, FL, during the springs of 1993 and 1994.

Aphid Species	Spring[1]		Known Vector of[2]:		
	1993	1994	WMV-2	ZYMV	PRSV-W
Acyrthosiphon kondoi Shinji	2	1	+	+	-
Acyrthosiphon pisum (Harris)	0	1	+	+	+
Anoecia sp.	0	3	-	-	-
Aphis craccivora Koch	29	12	+	+	+
Aphis fabae Scopoli complex	3	5	+	-	+
Aphis gossypii Glover	119	52	+	+	+
Aphis helianthi Monell complex	0	3	-	-	-
Aphis middletonii (Thomas)	137	97	+	+	-
Aphis nerii Boyer de Fonscolombe	0	2	+	-	+
Aphis spiraecola (=A. citricola) Patch	25	50	+	+	+
Aphis sp.	1	3	-	-	-
Aulacorthum solani (Kaltenbach)	1	0	+	-	+
Brachycaudus helichrysi (Kaltenbach)	13	7	-	-	+
Capitophorus elaeagni (Del Guercio)	2	2	-	-	-
Hayhurstia atriplicis (L.)	3	7	-	-	-
Hyalopterus pruni (Geoffroy)	0	2	+	-	+
Hyperomyzus lactucae (L.)	18	3	-[3]	-	-
Hysteroneura setariae (Thomas)	1	0	+	-	-
Lipaphis erysimi (Kaltenbach)	22	41	-	+	+
Macrosiphum euphorbiae (Thomas)	6	13	+	+	+
Sitobiun avenae (Fabricius)	0	12	-	-	-
Myzocallis discolor (Monell)	1	0	-	-	-
Myzus persicae (Sulzer)	15	113	+	+	+
Phylloxera sp.	0	5	-	-	-
Pleotrichophorus sp.	4	1	-	-	-
Rhopalosiphum cerasifoliae (Fitch)	1	0	-	-	-
Rhopalosiphum maidis (Fitch)	7	3	+	-	-
Rhopalosiphum nymphaeae (L)	0	1	-	-	-
Rhopalosiphum padi (L.)	7	14	+	-	-
Rhopalosiphum rufiabdominalis (Sasaki)	9	16	-	-	-
Schizaphis graminum (Rondani)	3	16	-	-	-
Sipha flava (Forbes)	0	3	-	-	-
Tetraneura nigriabdominalis (Sasaki)	170	208	-	-	-
Therioaphis riehmi (Boerner)	5	3	-	-	-
Therioaphis trifolii (Monell)	4	2	-	-	-
Uroleucon pseudambrosiae (Olive)	137	1390	+	-	+
Uroleucon sonchellus (Monell)	0	2	-	-	-
TOTAL[4]	**745**	**2093**	**16**	**9**	**13**

[1] Total aphids caught in 32 traps distributed in four plots from 16 April to 21 June in 1993 and from 21 March to 17 June in 1994.

[2] Known vector species for WMV-2 = watermelon mosaic virus, ZYMV = zucchini yellow mosaic virus, and PRSV-W=papaya ringspot virus type W (Tables 2.2-2.4 for appropriate references)

[3] DeSa and Kitajima (1991) reported transmission of WMV-2 by one unidentified species of *Hyperomyzus*.

[4] Accumulated counts per year and total number of species known to be vectors for each virus.

Model development: Stage 1

Correlation matrix. The correlation matrix was inspected separately for each year to identify potential high partial correlations (Tables 4.2 and 4.3). In 1993, with the exception of the correlation between *Ac* and *Am* (r=0.63, p=0.0001), partial correlations were, in general, small ($r \leq 0.58$) (Table 4.2). The Pearson's correlation coefficients (i.e., r-values) were taken into account more than the significance values (i.e. p-values). P-values were significant in most of the partial correlations because the high number of observations regressed (n=53). The variable, *Up* generally had the lowest r-values with all other variables ($r \leq 0.21$), suggesting that this variable should be associated closely with a single principal component.

Table 4.2 Correlation matrix of seven aphid vectors of WMV-2 and/or ZYMV caught in green tile water pan traps from 16 April to 21 June 1993.

Variables	*Ag*	*Am*	*As*	*Mp*	*Up*
Ac	0.3478 [1] 0.0107 [2]	**0.6280** 0.0001	0.1708 0.2213	0.3252 0.0175	-0.0198 0.8879
Ag		0.5550 0.0001	0.1976 0.1561	0.3527 0.0096	0.5759 0.0001
Am			0.3650 0.0072	0.4597 0.0005	0.2103 0.1305
As				0.2663 0.0539	0.1005 0.4737
Mp					0.0506 0.7189

[1] Pearson correlation coefficients associated with paired variables. *Ag* = *Aphis gossypii*, *Ac* = *A. craccivora*, *Am* = *A. middletonii*, *As* = *A. spiraecola*, *Mp* = *Myzus persicae*, and *Up* = *Uroleucon pseudambrosiae*.
[2] Prob > |R| under Ho: Rho=0 / N = 53.

In 1994, partial correlation values were also generally small ($r \leq 0.40$) (Table 4.3). The variable, *Am,* had the highest r-value with variables *Ag* and *Mp* (r = 0.40 and 0.39, respectively), thus some reduction of data dimensionality may be possible. The remaining variables had very low partial correlations. The variable, *Up* had, in general,

the lowest r-values compared with all other variables ($r \leq 0.15$), indicating possible strong association with a single principal component.

Table 4.3 Correlation matrix of six aphid vectors of WMV-2 and/or ZYMV caught in green tile water pan traps from 21 March to 17 June 1994.

Variables	*Ag*	*Am*	*As*	*Mp*	*Up*
Ac	0.1077 0.3968	-0.1366 0.2815	-0.2192 0.0818	-0.0978 0.4416	0.1272 0.3164
Ag		0.4047 0.0009	-0.1254 0.3232	-0.0787 0.5362	0.1485 0.2415
Am			-0.0663 0.6026	0.3948 0.0012	0.0097 0.9388
As				0.2300 0.0674	0.0050 0.9684
Mp					-0.0466 0.7147

[1] Pearson correlation coefficients associated with paired variables. *Ag = Aphis gossypii*, *Ac = A. craccivora*, *Am = A. middletonii*, *As = A. spiraecola*, *Mp = Myzus persicae*, and *Up = Uroleucon pseudambrosiae*.
[2] Prob > |R| under Ho: Rho=0 / N = 64.

Selection of principal components. Independent selection of principal components per year was defined at a cut-off value of $\lambda \geq 1.0$ (Hawkins and Fatti, 1984). In 1993, the first two principal components (PC) were selected (PC1 and PC2) to explain a cumulative variance of 64.4% of the overall data set (Table 4.4). In 1994, the first three PC's were selected (PC1-PC3) to explain a cumulative variance of 67.4% (Table 4.5).

Table 4.4. Eigenvalues and partial and cumulative proportion of variance explained by six principal components based on the correlation matrix of data set 1993.

Principal component	Eigenvalue (λ)	Eigenvalue difference	Partial variance	Cumulative variance
PC1	2.63703	1.40615	0.439504	0.43950
PC2	1.23087	0.36221	0.205146	0.64465
PC3	0.86866	0.19328	0.144777	0.78943
PC4	0.67538	0.36932	0.112564	0.90199
PC5	0.30606	0.02407	0.051010	0.95300
PC6	0.28199	.	0.046999	1.00000

Table 4.5. Eigenvalues and partial and cumulative proportion of variance explained by six principal components based on the correlation matrix of data set 1994.

Principal component	Eigenvalue (λ)	Eigenvalue difference	Partial variance	Cumulative variance
PC1	1.55615	0.080685	0.259359	0.25936
PC2	1.47547	0.458271	0.245911	0.50527
PC3	1.01720	0.128413	0.169533	0.67480
PC4	0.88878	0.173935	0.148130	0.82293
PC5	0.71485	0.367292	0.119141	0.94207
PC6	0.34756	-	0.057926	1.00000

Using the criterion of Jolliffe, of a cut-off value at $\lambda \geq 0.7$ (Jolliffe, 1986), at least one additional principal component could be selected in each data set (PC3, $\lambda = 0.87$ in 1993, and PC4, $\lambda = 0.89$ in 1994), thus increasing the cumulative variance to 78.9% (1993) and 82.2% (1994); however, the small number of variables in the original data set precluded consideration of that possibility.

Major and minor principal components. An initial selection of variables was done independently for each year by combining the criteria of major and minor principal components.

In 1993, the variable most clearly associated with a major principal component (those with $\lambda \geq 1$) was *Up*, due to the highest absolute weight (0.7585) on PC2 (Table 4.6). Although *Am* had the highest weight on PC1 (0.5327), it also had the highest absolute weight on a minor PC (PC6) (0.6867), so that further analyses (i.e., varimax rotation and biplots) were used to interpret the effect of this variable on the overall variance. Similar assertions can be made about *Ag*. Variables that could clearly be eliminated due to their stronger association with a minor PC were *As* (PC3), *Mp* (PC4), and *Ac* (PC5) (Table 4.6).

The cause for elimination of *As* and *Mp* could be due to their relatively small contribution to the overall variance (less than 14.4 and 11.2%, respectively), whereas *Ac* could be due to its correlation with other variables (correlation matrix, Table 4.2).

Table 4.6. Absolute weights of eigenvectors associated with two major and four minor principal components based on the correlation matrix of the 1993 data.

Variable	Major principal components[1]		Minor principal components			
	PC1	PC2	PC3	PC4	PC5	PC6
Ag	0.48185	0.40000	0.14611	0.05245	0.49018	0.58603
Am	**0.53276**[2]	0.15857	0.11297	0.18928	0.41329	**0.68674**
Mp	0.39244	0.26114	0.09878	**0.84928**	0.21567	0.01537
Up	0.26355	**0.75858**	0.05014	0.03172	**0.52072**	0.28358
As	0.30008	0.16115	**0.87058**	0.30467	0.07176	0.16759
Ac	0.41285	0.38109	0.44238	0.38250	**0.51584**	0.27605

[1] Principal components (PC's) with eigenvalues ≥ 1 ($\lambda \geq 1$) were defined as major PC's, principal components with $\lambda \leq 1$ were defined as minor PC's.
[2] Bold numbers represent the highest absolute weights for each principal component.

In 1994, the variables *Ag* and *Up* were clearly associated with the major PC's two and three (PC2, PC3) with weights of 0.5949 and 0.8222, respectively. Thus these variables contribute significantly to the overall variance of the data. On the other hand, the variable *Ac* could be eliminated due to its highest weight (0.7201) being on the minor PC four (PC4) (Table 4.7).

Table 4.7. Absolute weights of eigenvectors associated with three major and three minor principal components based on the correlation matrix of the 1994 data.

Variable	Major principal components			Minor principal components		
	PC1	PC2	PC3	PC4	PC5	PC6
Ag	0.29888	**0.59497**	0.00373	0.34146	0.47273	0.46537
Am	**0.67235**[1]	0.27336	0.16043	0.01548	0.10982	**0.65967**
Mp	0.57375	0.22751	0.11424	0.57457	0.15460	0.50195
Up	0.02190	0.31991	**0.82224**	0.12213	0.45373	0.01705
As	0.20559	0.49373	0.51265	0.13953	**0.61697**	0.22574
Ac	0.29438	0.41633	0.14930	**0.72016**	0.39245	0.21225

[1] Principal components (PC's) with eigenvalues ≥ 1 ($\lambda \geq 1$) were defined as major PC's, principal components with $\lambda \leq 1$ were defined as minor PC's.
[2] Bold numbers represent the highest absolute weights for each principal component.

The underlying cause for elimination of this variable could be due not to the correlation with other variables (correlation matrix, Table 4.3), but rather due to its

irrelevant contribution in characterizing the data structure. A look at the original data confirms this conclusion, because counts for this aphid were, in general, zero. The remaining variables, *Am, Mp,* and *As* can not be selected clearly because of their similar weights in both a major and a minor PC. Thus, further analyses were used for their interpretation.

Varimax rotation of major PC's. Varimax rotation of major PC's was done independently with the data for each year. In both years, varimax rotation helped to verify and improve previous selection of putatively important variables. In 1993, the overall variance of FACTOR1 seemed to be determined largely by *Am* and *Ac*, and FACTOR2 was defined mostly by *Up* and *Ag*, as indicated by their highest absolute weights for these variables (Table 4.8). Thus, this analysis verified the importance of *Up* as suggested by the criteria of major and minor PC's and also allowed clear selection of *Am* and *Ag* at this step. It failed, however, to verify the spurious attribute of *Ac* as suggested by inspection of the minor PC5 (Table 4.6). Further analysis (i.e. the biplot display) was used to qualify this variable.

Table 4.8. Varimax rotated eigenvectors associated with two (1993) and three major principal components (1994).

	1993		1994		
Variables	**FACTOR1**[1]	**FACTOR2**	**FACTOR1**	**FACTOR2**	**FACTOR3**
Ag	0.44465	**0.78199**	**0.72953**	-0.26294	0.24492
Am	**0.83313**[2]	0.29212	**0.87889**	0.23867	-0.10253
Mp	0.69574	0.07766	0.36330	0.68099	-0.07873
Up	-0.06360	**0.94203**	0.06837	0.02302	**0.91338**
As	0.51010	0.09610	-0.26016	**0.76316**	0.20683
Ac	**0.79238**	-0.01965	-0.00166	-0.52136	0.37612

[1] Varimax rotation was performed only with major principal components ($\lambda \geq 1$).
[2] Bold numbers represent the highest weights for each factor.

In 1994, the overall variance of FACTOR1 was determined largely by *Ag* and *Am*, FACTOR2 was defined mostly by *As*, and FACTOR3 was determined primarily by *Up*, as indicated by their highest absolute weights for these variables (Table 4.8).

Thus, this analysis verified the importance of *Ag* and *Up* as suggested by the criteria of major and minor PC's and also allowed clear selection of *Am* and *As* at this step.

Biplot displays. Biplot displays of varimax rotated major PC's were inspected independently for each year. In 1993, the biplot of PC1 vs. PC2 (FACTOR1 vs. FACTOR2) showed that *Am* represented the longest vector (in a geometrical sense, i.e., lines) in direction to PC1 (Figure 4.2). The relatively small angles between that variable and *Ac*, *Mp*, and *As*, suggested some degree of positive correlation among them. These correlations are better estimated for those vectors more adequately represented in the planar display, i.e., *Am* vs. *Ac* (see the correlation matrix, Table 4.2). Thus only *Am* could be selected to represent PC1. The variable *Up* was clearly the longest vector and was more parallel to PC2; therefore this variable could also be selected. Although *Ag* was not parallel to any of the axes, its length suggests that this variable should also be considered to explain the overall variance comprised by this biplot, i.e., 64.4%.

In 1994, the biplot of PC1 vs. PC3 (FACTOR1 vs. FACTOR3) showed that *Am* and *Ag* represented the longest vector in direction to PC1. However, the relatively small angle between them suggests that only the longer and more parallel vector to PC1 should be selected, i.e., *Am*. The variable *Up* could be selected to represent PC3 because it is associated clearly with that PC (Fig. 4.3A). The biplot of PC2 vs. PC3 (FACTOR2 vs. FACTOR3) shows that *As* is the longest vector associated with PC2. The variable *Up* was again clearly associated with PC3 (Fig. 4.3B). Thus, *Up*, *Am*, and *As* were selected.

Model development: Stage 2

Selection of lagged variables. The variables *Up*, *Am*, and *Ag* in 1993, and *Up*, *Am*, and *As* in 1994, transformed as scores of principal components, were lagged with respect to the highest change of disease incidence. Thirty lagged variables for the

1993 data and 32 for the 1994 data were inspected regarding their capability to correlate with changes in disease incidence. The number of lagged variables was initially narrowed down to seven (*Up 1-1, Up 1-4, Am 2-1, Am 2-7, Am 2-8, Ag 3-2,* and *Ag 3-1*) for 1993 and eight (*As 1-1, Am 2-1, Am 2-2, Am 2-7, Am 2-8, Up 3-1, Up 3-2,* and *As 1-1 x Am 2-8*) for 1994.

Regression models. The best models generated for the different combinations of lagged variables are indicated in the Table 4.9.

Table 4.9. Coefficient of determination (R^2), significance of R^2-values, and Mallow's C_p for multiple correlation models between change of disease incidence, as dependent variable, and several combinations of lagged counts of aphid vector species being used as independent variables in 1993 and 1994. Leesburg, FL.

Model	Independent variables[1]	R^2	Prob > F	C_p[2]
	Year: 1993	**Dependent variable:** $\Sigma (Y_t - Y_{t-1})$[3]		
$y_{1\text{-}93}$	*Up1-4*	0.54	0.0068	-0.98
$y_{2\text{-}93}$	*Ag3-2*	0.47	0.0065	13.92
$y_{3\text{-}93}$	*Up1-4, Am2-7*	0.59	0.0291	2.67
$y_{4\text{-}93}$	*Ag3-2, Am2-8*	0.66	0.0027	7.58
$y_{5\text{-}93}$	*Ag3-2, Am2-8, Up1-1*	0.82	0.0005	2.38
	Year: 1993	**Dependent variable:** arc sine $[\Sigma (Y_t - Y_{t-1})]^{1/2}$		
$y_{6\text{-}93}$	*Ag3-2, Am2-8, Up1-1*	0.70	0.0104	2.39
	Year: 1994	**Dependent variable:** $\Sigma (Y_t - Y_{t-1})$		
$y_{1\text{-}94}$	*As 1-1 x Am 2-8,*	0.84	0.0001	12.20
$y_{2\text{-}92}$	*As 1-1 x Am 2-8,* Up3-2	0.94	0.0001	1.00
	Year: 1994	**Dependent variable:** arc sine $[\Sigma (Y_t - Y_{t-1})]^{1/2}$		
$y_{3\text{-}93}$	*Up3-2, Up 3-1*	0.60	0.0173	1.56
$y_{4\text{-}94}$	*Up3-2, Up 3-1, Am2-8, As1-1*	0.90	0.0099	3.27
	Year: 1994	**Dependent variable:** $[\Sigma (Y_t - Y_{t-1}) /4]$		
$y_{5\text{-}95}$	*As 1-1 x Am 2-8,* Up3-2	0.94	0.0001	1.00

[1] *Ag = Aphis gossypii, Am = A. middletonii, As = A. spiraecola,* and *Up = Uroleucon pseudambrosiae.* Numbers associated with each aphid represent the lagged function with respect to disease incidence at any given time.

[2] Mallow's C_p-values similar to the number of parameters in the model indicate less bias of estimates of the dependent variable.

[3] Change in percent of disease incidence averaged $[(Y_t - Y_{t-1}) / 4]$ and accumulated per the four plots per year $[\Sigma (Y_t - Y_{t-1})]$. The arc sine of $[\Sigma (Y_t - Y_{t-1})]^{1/2}$} is a transformation for normality.

The most appropriate models were, in general, obtained with change of disease incidence accumulated per the four plots per year ($\Sigma [Y_t - Y_{t-1}]$) used as the dependent variable. Figure 4.4 shows that changes in disease incidence were similar among plots in 1994 but differed to some extend in 1993, regardless of the fact that the average apparent infection rates were statistically similar (Chapter 3). Thus, models based on changes in disease incidence accumulated for the four plots per year were emphasized in this study.

In 1993, there were no one-independent variable models with R^2-values higher than 0.55 and $C_p \leq p$. The two-variable models had fairly high R^2-values (0.59 and 0.66), however, with one model only one of the two regression parameters was significant, and the other model had a high C_p-value (7.58). Only the three-variable models seemed to have a more stable structure. The variables *Ag3-2, Am2-8, Up1-1* explained the changes in disease incidence better as accumulated values than the transformed version of the dependent variable (i.e., arc sine $[\Sigma (Y_t - Y_{t-1})]^{1/2}$). This model can be written as $\hat{y}_{5-93} = 0.09Up + 0.10\ Am + 0.11\ Ag$, in which $\hat{y}_{5-93}$ represents the prediction of change in disease incidence as $\Sigma (Y_t - Y_{t-1})$ at any given time; *Up* is the number of *Uroleucon pseudambrosiae* individuals trapped at the 21st day before the disease assessment and transformed as PC-scores; *Am* (*Aphis middletonii*) and *Ag* (*A. gossypii*) are PC-scores of counts of individuals in the 35th and 32nd day, respectively, before the disease assessment was made.

The progression of observed vs. predicted values of accumulated changes in disease incidence over time are given in Figure 4.6. The precision of estimates with this model was 82% (p=0.0005), all regression parameters were significant ($p \leq 0.021$), and the Mallows's C_p value was close to the number of parameters in the model (Cp = 2.38).

In 1994, there were no single independent variable models with R^2-values relatively high, so that they were not included in Table 4.9. There was a model built with

the product of As and Am as a single independent variable with relatively high R^2-value. This model, however, had a high Mallow's C_p value (C_p = 12.20). Three models with at least three independent variables had R^2- values $\geq$ 0.90 and acceptable Mallow's C_p-values (Table 4.9). Two of these three models differed only in the type of dependent variable being estimated (i.e., $\Sigma [Y_t - Y_{t-1}]$ and $\{\Sigma [Y_t - Y_{t-1}] / 4\}$). The remaining model, which included four independent variables, had a variable (*Up*) with two lagged periods and one out of four regression parameters was not statistically significant. Therefore this model was not considered the most suitable.

Because in 1993, the most appropriate model was generated with the accumulated change in disease incidence, the model with this type of dependent variable was selected for the 1994 data for the purpose of comparison. That is: $\hat{y}_{2-94} = 0.14Up + 0.12\ (Am)(As)$, in which $\hat{y}_{2-94}$ represents the prediction of change in disease incidence as $\Sigma\ (Y_t - Y_{t-1})$ at any given time; *Up* is the number of *U. pseudambrosiae* individuals trapped at the 12th day before the disease assessment and transformed as PC-scores; *Am* (*Aphis middletonii*) and *As* (*A. spiraecola*) are PC-scores of counts of individuals at the 22nd and 24th day before the disease assessment was made.

The progression of observed vs. predicted values of accumulated changes in disease incidence over time are given in Figure 4.5. The precision of estimates with this model was 94% (p=0.0001), all regression parameters were significant ($p \leq 0.003$), and the Mallows's C_p value was close to the number of parameters in the model (C_p= 1).

Discussion

Abundance and species composition. Based on the literature (Chapter 2: Tables 2.2-2.4), previous studies in Central Florida had identified a total of 15 aphid species known to be vectors of WMV-2, 8 of ZYMV, and 12 of PRSV-W (Adlerz, 1987,

1978b; 1974a, Webb et al., 1994; Webb and Kok-Yokomi, 1993). Among these aphid vectors, only *Aphis illinoisensis* (WMV-2) was not detected in this study. Conversely, *Acyrthosiphon kondoi* Shinji, a known vector of at least one of these viruses and not previously trapped in Florida, was caught in 1993 (2 individuals). The low numbers of aphids trapped could explain the lack of detection of this species in previous studies and vice versa (Webb et al., 1994; Adlerz, 1987, 1978b; 1974a).

The total number of aphid species known to transmit WMV-2, reported in Florida, increased from 15 to 16. This represents about 38% (16/42) of the total species of WMV-2 vectors reported worldwide (Chapter 2: Table 2.2, references included). The total number of vector species of ZYMV increased from 8 to 9, about 69% (9/13) and of PRSV-W remained the same, about 50% (12/24) of the total species reported worldwide for these two viruses. Whether or not these aphid vectors play a major role in spreading the viruses at the field level is the key factor in epidemiological studies (Mora-Aguilera et al., 1993a; 1992; Madden et al., 1990; Madden et al., 1987c; Clement et al., 1986; Marcus and Raccah, 1986; Sigvald, 1986; Ruesink and Irwin,1986; Watson and Healy, 1953). The approach used to address this aspect in this study was, an initial selection of species based upon abundance and population dynamics, a further reduction of the number of species through a series of multivariate methods, and finally, a selection of species based on their degree of association with changes in disease incidence.

Regarding the initial selection of species, at least two aspects, perhaps, should be considered. These are the type of assay used in transmission studies from which we obtain information about transmission rates, and the field trapping method, because it may affect both estimates of population density and species composition (Webb et al., 1994; Boiteau, 1990; Taylor and Palmer, 1972).

Most studies of transmissibility of WMV-2, PRSV-W, and ZYMV have been conducted as controlled access tests with single or multiple apterae (DeSa and

Kitajima, 1991; Purcifull et al., 1984a; Yamamoto et al., 1982; Lecoq et al, 1981; Tewari, 1976; Karl and Schmelzer, 1971; Coudriet, 1962). Therefore, one must take into account that behavioral aspects, and genetic diversity of the aphid, virus, and the host are not fully considered. Arena tests and field live assays have been conducted in the cucurbit-potyviruses system to incorporate at least one of these aspects in studies of transmissibility (Webb and Kok-Yokomi, 1993; Adlerz, 1987; Castle et al., 1992). These type of assays, particularly the field live studies, suggest that only a few aphid species play a major role in spreading virus diseases at the field level (Adlerz, 1987; Castle et al., 1990). Adlerz (1987) found, in a three-year study in Central Florida, that only 4 out of 40 species (46 individuals out of 1252) transmitted WMV-2, ZYMV, or both, but none transmitted PRSV-W to squash. Similarly, Castle et al. (1990), determined that only 4 out of 33 species (44 individuals out 18,837) transmitted WMV-2 or ZYMV in muskmelon fields in southern California.

Webb et al. (1994), in assessing the effect of trap color on abundance and species composition of alate aphids caught in Central Florida, suggested that the trapping method would affect one's conclusions about which aphid could be more important. They suggested that the role of *U. pseudambrosiae* in epidemics of WMV-2 and/or PRSV-W may have been overlooked in previous studies (Adlerz, 1987;1974a), whereas the importance of *A. spiraecola* may have been overemphasized because of its strong attraction to yellow. To prevent potential misleading information, Webb and coworkers (1994) suggested the green tile water pan trap, because its spectral reflectance matched that of green leaves, to estimate abundance and species composition of alate aphids landing in the watermelon crop. Earlier studies also suggested this type of trap for epidemiological studies in soybean (*Glycine max*) and potato (*Solanum tuberosum*) (Boiteau, 1990; Irwin and Schultz, 1981).

The use of green tile traps in this study proved to be adequate for correlative studies of aphid vectors with changes in disease incidence. However, preliminary trials

to define the optimum number of traps should perhaps be done before designing experiments. The use of five traps within a 1.5-ha watermelon field in 1992 seemed to trap mostly aphid species that land in great numbers (15 species total) (Webb et al., 1994), whereas the 32 traps per plot used in this study resulted in the capture of some apparently less frequently landing species (37 species total). Because vector species with higher landing rates are often characterized as the most important in spreading disease (Castle et al., 1990; Adlerz, 1987; Yamamoto, 1980), a reasonably small number of traps may not represent a limiting factor for some epidemiological studies. However, a trade-off of time, costs, and purpose of trapping should be considered in selecting number as well as the type of traps (Webb et al., 1994; Taylor and Palmer, 1972) .

Model development. Concerning the final selection of vector species through regression analyses, an important drawback inherent to almost all correlative studies involving a set of variables being used as independent repressors is the problem of multicollinearity (Mora-Aguilera et al., 1993; Chuang and Jeger, 1987; Jolliffe, 1986; Dunteman, 1989). In this study, the approach used to account for this problem was an integration of several multivariate techniques as an initial stage in the development of models. This includes the combination of the criteria of minor and major PCs (Hawkins and Fatti, 1984), inspection of varimax rotated major PC's (Jolliffe, 1989), and biplot displays of varimax rotated major PC's (Gabriel,1971). This study suggests that several, rather than one individual technique could be used to eliminate spurious variables with reasonable confidence. For example in 1993, *Ac* was apparently detected as an important variable with the criteria of varimax rotated major PC's when, in fact, it was detected as a potential spurious variable with the correlation matrix (Table 4.2) and clearly identified as such with the criteria of major and minor PC's (Table 4.6) and the biplot display (Figure 4.1). Similar results were also observed by Mora-Aguilera

et al. (1995) in a comparative study of epidemics of PRSV-P based on nine curve parameters used as variables.

By integration of these multivariate techniques, not only multicollinearity could be reduced, but additional benefits could be achieved: 1) variables with low contribution to the overall variance and not necessarily correlated with others can be eliminated (Dunteman, 1989). This seemed to be the case with *Mp* and *As* for 1993 and *Mp* and *Ac* for 1994 in this study. Elimination of variables at this stage prevent their effect on the regression parameter estimates ($\hat{\beta}\, i$) in the model (Dunteman, 1989). Also, because of the usual rather small total degrees of freedom, it allows testing of more lag-periods for each putatively important independent variable; and 2) it enables the use of principal components, as independent variables, in multiple regression analysis (Dunteman, 1989, Jolliffe, 1986). This has the benefit of using a standardized set of new variables representing an orthogonal multivariate space (i.e. uncorrelated variables) (Jolliffe, 1986), provided that they are obtained only with variables previously selected. In this study, the initial selection of *Up, Ag*, and *Am* in 1993 and *Up, As, Am*, in 1994 before the regression analyses, seemed to be adequate since all of these variables were included in the best model for each year. Also, two aphid species were repeatedly included in these two best models. Yet, the interpretation of a combined approach of principal component and multiple regression analysis should be done carefully. A discharged variable with PCA may in fact be correlated to a dependent variable (Dunteman, 1989; Jolliffe, 1986).

In both years, *U. pseudambrosiae* and *A. middletonii* were identified as important vectors to describe changes in disease incidence induced by WMV-2 in 1993, and mostly ZYMV in 1994, in spring watermelon in Central Florida. *Aphis gossypii* in 1993, and *A. spiraecola* in 1994 were also identified as putatively important in the system studied. No single aphid species appeared to be the most important vector in the spring season in Central Florida as demonstrated by the fact that there was no

model with a single independent variable considered appropriate to describe disease incidence (Table 4.9). Similar findings, based on modeling and /or on laboratory and field assays, have been reported elsewhere for plant crop systems with potyviral diseases (Castle et al., 1990; Sigvald, 1986; Ruesink and Irwin, 1986; Marcus and Raccah, 1986; Nelson and Tuttle, 1969). In a few cases, however, a single vector species has been blamed as the major vector in the field (Yamamoto et al. 1986; Tewari, 1976).

Although the exact biological meaning of both lagged periods and magnitudes of the regression parameters can not be easily established, and perhaps, never fully determined with this type of statistical approach, some interpretation can be made.

The estimated magnitudes of the regression parameters associated with the different aphid vector species were similar within a model (0.09, 0.10, 0.11 in 1993, and 0.14, 0.12 in 1994). Therefore, it can be suggested that the aphid species may have, on average, similar vector propensities [i.e., transmission rates under natural conditions (Irwin and Ruesink, 1986)] but different vector intensity [a product of vector propensity and numerical density (Irwin and Ruesink, 1986)] in disease spread. The major role of the different aphid species can, however, be played at different stages of the epidemic development. This is suggested by the fact that, in both years, *U. pseudambrosie* had a shorter lag period than *A. middletonii*.

In 1994, the year where the average apparent infection rates were higher (Chapter 3), the lag period was shorter for all aphids. For example the lag period for *U. pseudambrosiae* was reduced from 21 in 1993 to 12 days in 1994, whereas it was reduced from 35 days to 22 for *A. middletonii*. These results indicate that *U. pseudambrosiae* may play its major role in the secondary dispersion of the disease, either within a field or to adjacent fields. The greatest trapping of this aphid species was observed from 12-15 May in 1993, when the crop was about 50% fully grown, and 1-5 May in 1994 with the crop 35-40% grown. At this time, the wild host (*Lactuca*

graminifolia [Michaux]) of the aphid, relatively abundant in the field surrounding the plots, ended its cycle and died. In contrast, *A. middletonii*, which feeds on the roots of several members of the Compositae, Cruciferae, Rubiaceae, Chenopodiaceae and Umbelliferae families (Adlerz, 1978; Holman, 1974), may be more important in the primary dispersion. Early flights of this aphid may be stimulated by drying out or death of different hosts during the winter. In 1993, after a severe frost on 14-15 March killed weeds and some early-planted crops, almost 45% more individuals of this aphid were trapped in comparison with 1994.

The statement, based on this statistical approach, about the primary dispersion role of *A. middletonii* seems to be biologically validated by earlier studies of Adlerz in Central Florida (Adlerz, 1987; 1978b). He found, using field live assays, that *Am* was always the first viruliferous aphid trapped during the spring (1987). However, Adlerz assigned to this aphid a major role in secondary dispersion (Adlerz, 1978b). Although secondary transmission can not be ruled out for *Am*, and perhaps for any aphid vector provided availability of inoculum, the data of this study suggest that *Up* plays a major role in the secondary spread of WMV-2 / ZYMV during the spring season.

The suggested distinctive role of *A. middletonii* and *U. pseudambrosiae* agrees with their population fluctuations during the season (Fig. 4.4 and 4.5). *Am* appeared earlier than *Up* in both seasons. In 1993, the first infected plant was detected eight days after the highest *Am* peak and seven days before the highest flight activity of *Up* (Fig. 4.4). By the time of the highest peak of *Up* (12 May), disease incidence increased from 0.3% (one plant on 11 May) to 13% (24 May) 12 days later, a necessary adjustment due to the incubation period. In 1994, the highest peak of *Am* occurred 5 days after the first peak and 14 days before the second highest peak of *Up*. However, according to the lag-period statistically selected to represent *Up* in the model (12 days), it is the second peak of this species that has an effect on the secondary spread of disease in 1994. Field disease records also appear to validate this statement. For

example, by the time of the two flushes of the second peak of *Up* (1 May and 4 May) disease incidence increased from 8% (2 May) and 13% (5 May) to 86% (12 May) and 96% (16 May) about 12 days later. Clearly this proves that the main role of *Up* is in the secondary dispersion process, particularly the second peak of flight activity.

As in 1993, the highest flight activity for *Am* (20 April), and the first peak of *Up* (14 and 15 April) occurred when no single plant was infected in the field in 1994 (Figure 4.6). The first infected plant was detected (25 April) 5 and 11 days after the *Am* and *Up* peak, respectively. This observation may suggest a role of *Up* in primary dispersion of viruses in 1994. However, if this were the case, more plants could have been expected to be infected on 25 April considering that a total of 120 and 200 *Up* individuals were captured, about 12 days earlier, on the two days of major flight activity. This leads to an estimate of an aphid landing value of 18 and 30 individuals per plant on those two days (Webb *et al.*, 1994), a value which can cause a significant change in disease incidence even with a few viral sources present in the area. On the other hand, the estimated aphid landing value of *Am* was 1.35 individuals per plant during its highest flight activity, a value that appears to be more in accord with the level of disease observed.

The vector *Up* may have a localized flight activity from weeds surrounding the field with limited epidemiological effect when few or no infected plants are present within the field or in the immediate vicinity, which in fact appeared to be the case during the first highest peak in 1994. *Am* may arrive from greater distances, with its viruliferous state being independent of the presence of local or adjacent inoculum. Weed hosts, as well as fields of infected cucurbits encountered in their flight, may provide the source of inoculum. Perhaps this may explain why Adlerz did not find weeds infected with WMV-2 adjacent to the fields when the first infected plants were detected (Adlerz, 1974a; 1969). Thus, the importance of *Hibiscus rosa-sinensis* (Malvaceae), a potential perennial source of WMV-2 in Central Florida (Adlerz, 1969),

usually located far from the fields, should not be dismissed, as Adlerz suggested (1969). Four species of the Umbelliferae and five of the Compositae, families that apparently are common hosts of *Am* (Holman, 1972; Adlerz, 1978), have been proven to be potential sources of inoculum for WMV-2 in different regions (Edwardson and Christie, 1991). Other summer annual weeds reported naturally infected with WMV-2 in Florida include four members of the Leguminosae: *Crotalaria spectabilis* Roth, *Indigofera hirsuta* L., *Alysicarpus vaginalis* DC., *Lupinus* spp (Kucharek and Purcifull, 1989; Adlerz, 1969).

Although our data suggest the importance of primary infections on epidemic development, the apparently higher vector propensity of *Up* than *Am*, due to its greater numerical density in comparison to *Am*, particularly in 1994 (Tables 4.1-4.3), suggest that secondary dispersion is a definitive force in driving the epidemics.

The epidemiological driving force of secondary infection in spring in Central Florida may also be explained by the participation of other minor vector species. The statistical inclusion into the models of *Aphis gossypii* in 1993, and *A. spiraecola* in 1994, their lag periods, and their population fluctuation over time suggest this idea. Vector propensity may explain the important contribution of these vectors species in only one season. The season when these species were apparently important had an overall trapping proportion of 119:52 (*Ag*), and 50:25 (*As*) with respect to the other season.

Whereas *Up* has just recently been reported as a vector of WMV-2 (Webb and Kok-Yokomi, 1993) and ZYMV (Chapter 5) in Central Florida, and further biological studies at the field level have to be carried out to validate its major role in secondary dispersion, earlier studies biologically validate our results with respect to *Am* and *As* (Adlerz, 1987; 1978a; 1978b). Adlerz concluded that "in Central Florida, only two or three species (i.e., *A. citricola* [now known as *A. spiraecola*], *A. middletonii* and possibly *M. persicae*) are important in the spread of viruses in the field in the spring" (Adlerz,

1987, p. 92). He concluded, based on the fact that of all naturally viruliferous individuals of a three year study (46 out of 1252), 52% were *Aphis spiraecola* (23 out of 643 individuals) and 40% (19 out of 508) were *A. middletonii*, both transmitting WMV-2 and ZYMV. The remaining 8% of all viruliferous individuals were *Myzus persicae* (1 out of 90) transmitting ZYMV, and *A. illinoisensis* (3 out of 11), transmitting WMV-2 (Adlerz, 1987). Because *A. spiraecola* and *M. persicae* are heavily attracted to yellow (Webb et al., 1994; Taylor and Palmer, 1972), the trap where most individuals of these species were collected in the Adlerz studies (Adlerz, 1987; 1978b), the importance of these species may have been overemphasized as Webb and coworkers suggested (Webb et al., 1994). No apparent contribution of *A. illinoisensis* in the development of epidemics was suggested by this study, perhaps due to its low population density. No individuals of this species were trapped in the two spring seasons (Table 4.1), nor in a previous study that included the spring and fall seasons (Webb et al., 1994) even though these aphids were commonly found in a nearby vineyard (S.E. Webb, personal communication).

In the western United States, the vector scenario appears to be different, perhaps due to the dry and temperate conditions (Hander et al., 1993; Castle et al., 1992; Nelson and Tuttle, 1969; Coudriet, 1962). In southern California, Castle and coworkers found naturally viruliferous individuals of *Myzus persicae* (29 out of 8206 individual tested) and *Acyrthosiphon pisum* (11 out of 2430) with the viruses WMV-2 and ZYMV, and *A. kondoi* (3 out of 3759) and *Rhopalosiphum padi* (1/1113) with WMV-2. *A. middletonii* and *U. pseudambrosiae* were not trapped in that three year study (Castle et al.,1992).

In Arkansas, *A. craccivora* and *A. gossypii* prevailed over *M. persicae*, *Acyrthosiphon pisum,* and *Rhopalosiphum maidis* in fall squash infected with WMV-2 (Hander et al., 1993). The last three species were heavily trapped during the spring but no infected plants were recorded. No attempts were made to either statistically

correlate or assay these species in this study (Hander et al., 1993). In southwestern and central Arizona, *M. persicae* and *A. gossypii* are believed to be responsible for transmission of WMV-2 and cucumber mosaic virus in spring cantaloupes (Nelson and Tuttle, 1969; Coudriet, 1962). Although these studies were not designed as live assays, Coudriet (1962) found only *A. gossypii* to be naturally viruliferous with WMV-2.

In Japan, Yamamoto et al. (1986) attributed the spread of WMV-2 among cucurbit fields to at least 14 of 23 known aphid vectors occurring in that country. *Aphis gossypii*, however, was considered to play a major role in primary and secondary viral dispersion based on laboratory transmission tests and abundance in the fields.

This study shows that it is possible to associate aphid species populations with viral disease incidence in spring watermelon in Central Florida. As in early studies (Adlerz, 1978a; 1974a), a long delay period between the peak of major aphid vectors and spread of WMV-2 was observed in 1993. A shorter delay period was observed in 1994, a year when ZYMV was the most common virus detected serologically, and average apparent infection rates were higher (Chapter 3). Under those conditions, the overall statistical approach used in this study appeared to detect a better correlation between aphid species population peaks and changes of disease incidence. Low levels of inoculum may mask this type of correlation as suggested by Adlerz (Adlerz, 1974a; 1978a). Under such conditions, perhaps many aphid vectors rather than only those suggested in this study may be responsible for the overall epidemic outcome. Other known vectors, seemingly important vectors elsewhere (i.e., *M. persicae*, *R. padi*, *A. kondoi*, *A. craccivora*) are also present in Central Florida and their participation can not be ruled out under certain conditions (Hander et al., 1993; Castle et al., 1992; Nelson and Tuttle, 1969; Coudriet, 1962).

This study also shows that the level of success of a stochastic approach, i.e., regression models, is contingent upon the level of understanding of the basic components of the pathosystem, including regional or local knowledge of the

abundance and species composition of aphid vectors, colonizing species, sources of inoculum, and type of transmission. The two basic biological assumptions necessary for the application of multiple regression models were satisfied in this study. That is, the viral epidemics in watermelon were driven by noncolonizing alate species, and WMV-2 and ZYMV, the viruses responsible for the disease (Chapter 3), are nonpersistently transmitted.

Because the selection of variables and the determination of parameters in regression models are based on quantitative associations between changes of disease incidence and aphid species, environmental and biological factors such as incubation period, effect of host genotype in transmission, transmission rates, serial transmission, etc. (Heathcote, 1986), are masked processes. These biological features are constricted in the regression parameter and the lag periods associated with the aphid species selected. Consequently the epidemiological understanding of the system can only be approximated with this quantitative approach. A qualitative approach, however, may not grant a full epidemiological understanding of a biological system either. Integrative studies, broader in scope, will always optimize the level of knowledge acquired in the system under study.

Additional investigations, including validatory studies, should be done to assess the regression models generated in broader climatic conditions and levels of inoculum in Central Florida. Regional knowledge, as well as local knowledge, could ensure a better understanding of the main forces responsible for the build up of viral epidemics in spring watermelon. Thus, specific disease management strategies could be more effectively suggested to diminish the effect on yield.

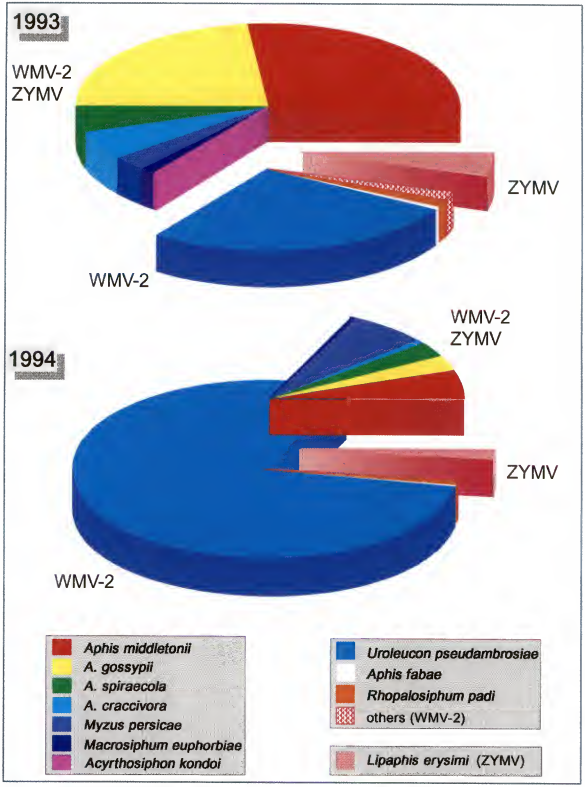


Figure 4.1. Proportion of aphids known to transmit WMV-2, ZYMV, or both caught in 32 green tile water pan traps from 16 April to 21 June in 1993 and from 21 March to 17 June in 1994. Total aphid vectors trapped: 512 for 1993 and 1774 for 1994. Total number of vectors species trapped: 14 for 1993 and 16 for 1994.

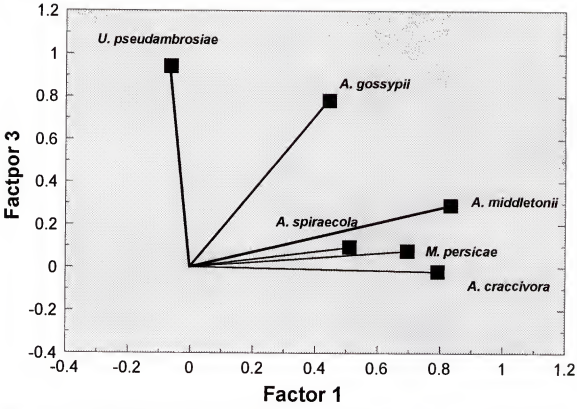


Figure 4.2. Varimax rotated biplots of factors 1 and 2. Lines represent aphid vectors of WMV-2 and ZYMV *Aphis middletonii*, *A. gossypii*, *A. spiraecola*, *A. craccivora*, *Myzus persicae*, and *Uroleucon pseudambrosiae*. Longest lines indicate variables that contribute most to explaining the variance comprised by the biplot. Small angles and angles of about 180° between two lines indicate positive and negative correlations, respectively, between variables associated with those lines.

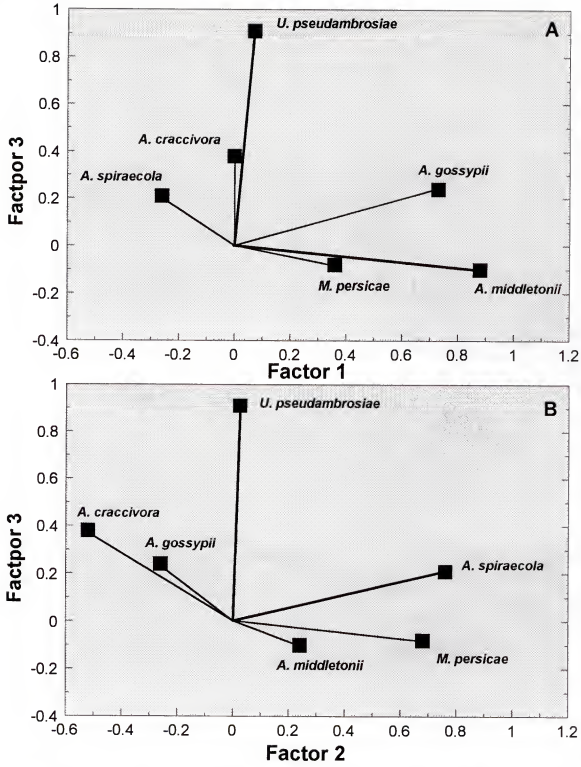


Figure 4.3. Varimax rotated biplots of factors 1 and 3 (A) and factors 2 and 3 (B). Lines represent aphid vectors of WMV-2/ZYMV: *Aphis middletonii, A. gossypii, A. spiraecola, A. craccivora, Myzus persicae, and Uroleucon pseudambrosiae*. Longest lines indicate variables that contribute most to explaining the variance comprised by respective biplot. Small angles and angles of about 180º between two lines indicate positive and negative correlation, respectively, between variables associated with those lines.

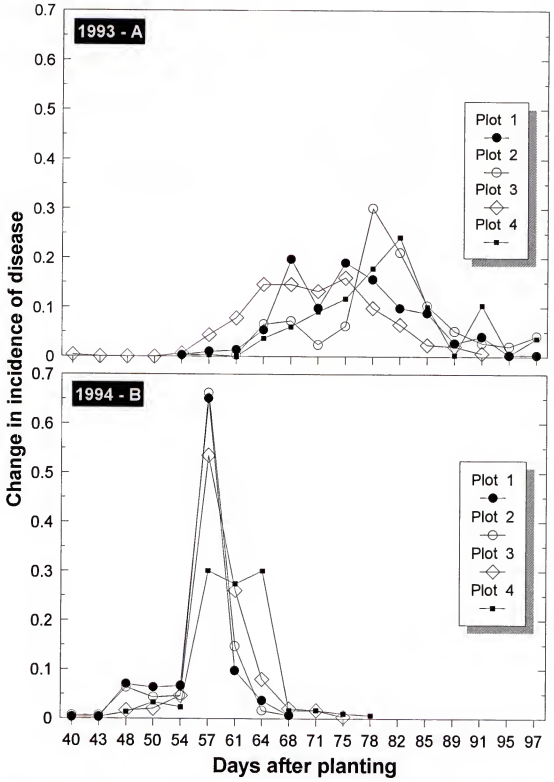


Figure 4.4. Change in the incidence of disease caused by watermelon mosaic virus (WMV -2) **(1993-A)**, and watermelon mosaic virus and zucchini yellow mosaic virus (ZYMV) **(1994-B)** in four experimental plots of spring watermelon (*Citrullus lanatus*) per year. Direct seeding was done on 24 March in 1993 and 14 March 1994. Leesburg, FL.

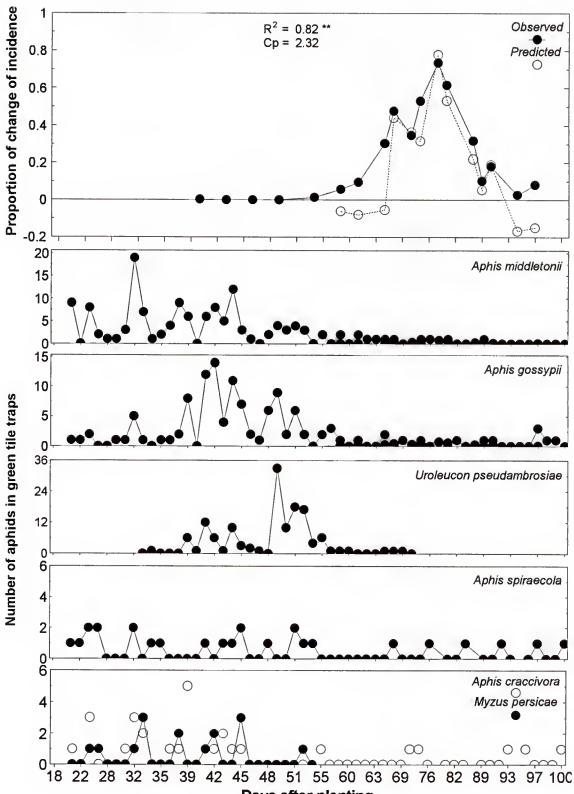


Figure 4.5. Observed and predicted changes of disease incidence (Y_t-Y_{t-1}) in watermelon with the model $Y = 0.09\ Up + 0.10\ Am + 0.11\ Ag$, and numbers of aphid vectors of WMV-2 / ZYMV *Aphis middletonii* (Am), *A. gossypii* (Ag), *A. spiraecola*, *A. craccivora*, *Uroleucon pseudambrosiae* (Up), and *Myzus persicae*. Spring 1993, Leesburg, FL.

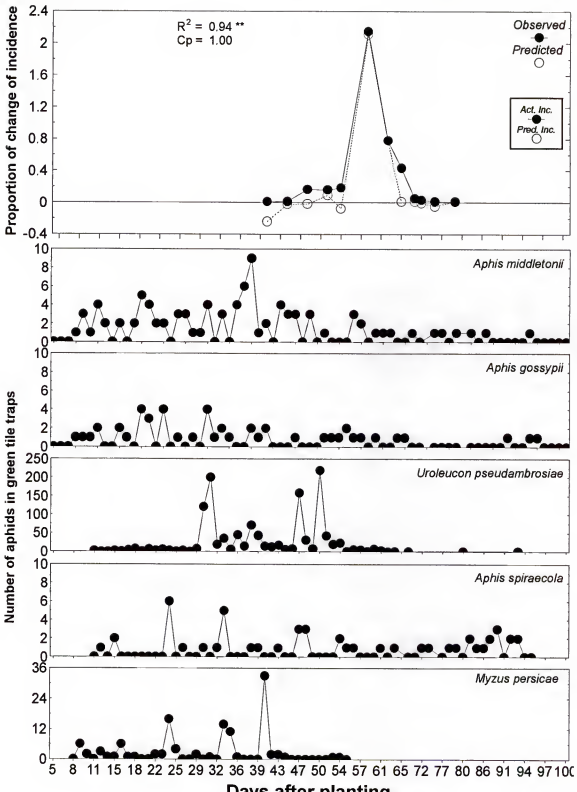


Figure 4.6. Observed and predicted changes of disease incidence (Y_t-Y_{t-1}) in watermelon with the model $Y = 0.14\,Up + 0.12\,(Am)(As)$ and numbers of aphid vectors of WMV-2 / ZYMV *Aphis middletonii* (Am), *A. gossypii*, *A. spiraecola* (As), *A. craccivora*, *Uroleucon pseudambrosiae* (Up), and *Myzus persicae*. Spring 1994, Leesburg, FL.

CHAPTER 5
TRANSMISSIBILITY OF ZYMV BY *UROLEUCON PSEUDAMBROSIAE* (OLIVE) (HOMOPTERA: APHIDIDAE), A PUTATIVE MAJOR VECTOR IN VIRUS EPIDEMICS.

Introduction

Epidemiological studies conducted in Central Florida during the spring of 1989 and 1990 (Webb and Linda, 1993) showed the possible involvement of a *Uroleucon* sp. in the acquisition and transmission of watermelon mosaic virus type 2 (WMV-2) to watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]. Many alate aphids of this species were caught in green tile traps, and two common weeds in the area, wild lettuce (*Lactuca graminifolia*) and spiny-leaved sowthistle (*Sonchus asper*), were heavily infested (Webb and Kok-Yokomi, 1993). The aphid was later identified as *Uroleucon pseudambrosiae* (Olive) and further research demonstrated its ability to transmit WMV-2 (Webb and Kok-Yokomi, 1993).

In 1994, spring virus epidemics in experimental plots of watermelon at Leesburg, Florida were mostly induced by an apparently accidental release of ZYMV from greenhouse to the field (Chapter 3). *U. pseudambrosiae* was implicated as a putative major vector of this virus, and perhaps of WMV-2, during secondary dispersion based on a series of statistical approaches, including principal component, varimax rotated biplots, and multiple regression analysis (Chapter 4). Thus, this study was conducted to biologically assess the ability of *U. pseudambrosiae* to transmit ZYMV in watermelon, which has not been reported before.

The determination of *U. pseudambrosiae* as a vector of ZYMV would support the hypothesis that this species plays a major role in secondary viral dispersion in Central Florida (Chapter 4). *Aphis middletonii*, the species that was suggested as a

major vector in primary dispersion, as well as *A. spiraecola*, a species with a minor role under field conditions (Chapter 4), have been previously shown to transmit both WMV-2 and ZYMV under field conditions in Central Florida (Adlerz, 1987, 1978b).

Although, in a previous study, Alderz obtained transmission of ZYMV under controlled access tests with a *Uroleucon* sp. trapped in the field (one aphid out of nine tested) (Adlerz, 1987), that result was not repeated with *U. pseudambrosiae* in preliminary controlled access tests with an isolate of ZYMV collected from watermelon in Leesburg, FL in 1991, even though this isolate was readily transmitted by *Myzus persicae* (Webb and Kok-Yokomi, 1993). In the present study, the transmissibility of ZYMV by *U. pseudambrosiae* was reassessed using, exclusively, the arena test methodology.

The arena test approach was developed to estimate *vector propensity* (*sensu* Irwin and Ruesink, 1986) without controlling the acquisition or inoculation access period (Halbert et al., 1994; Webb and Kok-Yokomi, 1993; Summer et al., 1990; Irwin and Ruesink, 1986). By incorporating the behavioral aspects of movement and probing duration and frequency, a more representative determination of efficiency of transmission could be achieved, and a closer resemblance to transmission under field conditions could be expected (Irwin and Ruesink, 1986).

Loss of ZYMV transmissibility with some aphid vectors has generally been observed after successive mechanical transfers of a virus to a susceptible host under controlled conditions (Lecoq and Purcifull, 1992), perhaps due to changes in the helper component or in the coat protein of the virus genome (Huet et al., 1994; Granier et al., 1993; Gal-On et al., 1992; Lecoq et al., 1991a; Lecoq, 1986). In this study, an isolate maintained in the greenhouse for 3.5 years from which the field isolate may have originated, and the field isolate itself, were compared in their capability of being transmitted by *U. pseudambrosiae*.

Material and Methods

Virus isolates. The zucchini yellow mosaic virus (ZYMV) isolate FC3326 (field isolate) was collected on 2 May 1994 from one of the first two watermelon plants, out of 300, that showed virus infection in an experimental plot at Leesburg, FL (Chapter 3). Single infection by ZYMV was confirmed with a sodium dodecyl sulfate (SDS)-immunodiffusion test (Purcifull et al., 1984a; Purcifull and Batchelor, 1977).

Additional viruses tested with SDS-immunodiffusion included the common viruses found in cucurbits in Florida, watermelon mosaic virus type 2, papaya ringspot virus type W, squash mosaic virus, cucumber mosaic virus, and an unnamed potyvirus (coded as 2932) (Purcifull et al., 1988; 1991). Plant tissue collected from the field was directly vacuum dried, ground and kept at 4°C until use. Symptoms of isolate FC3326 included strong yellowing, mosaic with some dark green vein banding, roughness, distortion of leaves and ends of runners, and severe necrosis of diseased tissue (see Plate 3.1, Chapter 3).

Freeze-dried pumpkin tissue infected with the isolate ZYMV-A58F91S (laboratory isolate) was provided by S. E. Webb (CFREC-Leesburg, FL). This isolate was collected in September 1991 from squash (*Cucurbita pepo* L.) grown in experimental plots at Leesburg, FL and was passaged mechanically an undetermined number of times. Identity of this virus was confirmed with ELISA (S. E. Webb, personal communication).

The WMV-2 isolate G2301 was collected from experimental plots of watermelon at Leesburg, FL in 1989 and was passaged by both mechanical and by aphid inoculations to pumpkin (*Cucurbita pepo* L., 'Small Sugar') plants (S. E. Webb, personal communication). The original sample provided by S. E. Webb for this experiment was freeze-dried tissue processed on 2 February 1991 and stored at about 4°C. Symptoms of this isolate included some leaf roughness and green vein banding, and mosaic which

were similar to those induced by the isolate FC3328 (an isolate collected in 1994) (Plate 3.1, Chapter 3).

Increase of inoculum. Because the inoculum provided was not enough to set up the entire series of experiments, the original inoculum was increased one time. Each isolate was separately inoculated in 70 seedlings of watermelon cv. 'Fiesta' (Northrup King Co, NK®, Gilroy, CA 95021) in the cotyledonary stage on 13 October 1994.

Inocula were prepared by mixing dried, pulverized tissue and Carborundum 600 in 0.02 M potassium phosphate buffer pH 7.5, until the consistency of a paste was obtained. Cheesecloth pads were dipped in inocula and rubbed on both sides of cotyledons. Inocula and buffer were kept on ice during the inoculation process. Inoculated plants were grown in a glasshouse at 25-30°C for two to three weeks and harvested upon development of symptoms on 27 October.

Tissue was vacuum-dried, ground, and kept in vacuum tubes in samples of about one gram. Tubes were labeled accordingly and stored at 4°C until needed. Precautions were taken at all stages during the increase of the inocula to prevent contamination of isolates.

Arena test.

The transmissibility of ZYMV-A58F91S and ZYMV-FC3326 by *U. pseudambrosiae* was determined with arena tests following the procedure described previously by Webb and Kok-Yokomi (1993). Because a synchronization of different experimental stages is required for the successful use of the arena test, the test will be described in a series of steps.

Step 1: Establishment of source and test plants. Seeds for transplants were germinated in an incubation chamber (Lab-Line Instruments, Melrose Park, ILL) at 37°C and 0:24 (L:D) photoperiod during three days. Sets of about 120 thiram-treated seeds of watermelon 'Fiesta' were distributed on the surface of a series of layers of moistened

paper. Trays with lids (30 x 13 x 6.5 cm) were used to prevent dehydration of seeds. Plants used both as viral sources and as test plants were transplanted 41 to 37 days before arena tests were set up (Table 5.1). Plants to be used as viral sources were transplanted in groups of 5-6 per pot (18 x 15 cm). Plants for arena tests were transplanted to 12-liter pots. Ten seedlings were transplanted in a circular pattern, about 2 cm from the edge of the pot.

Step 2. Inoculation of source plants. Virus isolates were inoculated individually on the same day to about 70 source plants. The time from inoculation to the establishment of transmission tests varied among experiments within a range of 9 to 32 days for ZYMV-A58F91S or 26 to 32 days for ZYMV-FC3326 and WMV-2-G2301 (Table 5.1).

Table 5. 1. Age of inoculum used in arena tests in three experiments of transmissibility of isolates ZYMV-FC3326, ZYMV-A58F91S and WMV-2-G2301 by *Uroleucon pseudambrosiae* and *Myzus persicae* and dates of test and source plant transplanting, inoculation of source plants, and set up of arena tests.

Exp.	Planting dates of [1]:		Inoc. date [2] of source plants	Days after [3] inoculation	Arena test set up
	Test plants	Source plants			
1	21 Oct.	21 Oct.	28 Oct.	32	29 Nov. 1994
2	26 Jan.	15 Jan.	26 Jan. , 12 Feb.	26 , 9	21 Feb. 1995
3	16 Feb.	16 Feb.	26 Feb. , 14 Mar.	31 , 15	29 Mar. 1995

[1] Watermelon cv. 'Fiesta' used for both test and source plants.
[2] ZYMV-3326 and WMV-2-G2301 were inoculated on 26 January (Experiment 2) and 26 February (Experiment 3), ZYMV-A58F91S was inoculated on 12 February (Experiment 2) and 14 March (Experiment 3).
[3] Time in days from inoculation of source plants to their use in the arena tests.

The inoculation procedure was performed as described before for the increase of inoculum. Two dried samples (about one gram) were used per isolate and experiment. Inoculated plants were incubated in a growth room at 22°C and 24-h

fluorescent light for experiment 1, and at 21°C and 12:12 photoperiod for experiments 2 and 3.

Stage 3. Aphid colonies. The stock aphid colonies used in this research have been maintained in Leesburg, FL for at least two years in a walk-in rearing room at 21 ± 2°C and a 16:8 (L:D) photoperiod. *M. persicae* and *U. pseudambrosiae* were reared in individual cages (100cm x 80cm x 80cm) on bell pepper (*Capsicum annuum* L.) and romaine lettuce (*Lactuca sativa* L.), respectively. Neither plant is a host of the viruses tested in this study (Edwardson and Christie, 1991).

At least 12 days before the transmission tests were established, apterous adults from the stock colony were transferred to two or three fresh host plants and then removed after 48 h to leave only nymphs on the host. About 50 adults per species were used to establish the colonies. These fresh colonies were reared at Gainesville, FL under laboratory conditions of about 22°C and 24-h light. Table 5.2 shows the approximate number of adults needed to produce enough offspring for an experiment consisting of 12 arena tests with 10 and 15 individuals of *M. persicae* and *U. pseudambrosiae* per test, and the number of days required for each species to produce mature aphids.

Table 5. 2. Approximate number of parents and time in days needed to produce the number of adults required for one experiment consisting of 12 arena tests for each aphid species.

Species	[1]Number of parents	[2]Progeny per aphid day^{-1}	Generation time to maturity (days)	Total aphids per experiment
U. pseudambrosiae	35.29	5.1	12	180
M. persicae	27.91	4.3	7	120

[1] Number of adult apterae of *U. pseudambrosiae* placed on healthy romaine lettuce and of *M. persicae* placed on healthy bell pepper.

[2] Progeny and generation time per species estimated with aphids reared at 21 ± 2°C and a 16:8 (L:D) photoperiod (S.E. Webb, unpublished).

Stage 4. Selection of source leaves. The third youngest leaf was preferentially taken from each source plant to be used as the source of the virus in the transmission tests. When symptoms were not well developed or necrosis prevented the use of this leaf, the second (10 times overall) or the fourth (5 times) youngest leaf was used. Only one leaf was selected per source plant. Symptoms of each leaf selected were recorded, and the plant from which the leaf was detached was tagged.

Selected leaves were detached from the source plants the night before the transmission tests were established and placed in 4-ml test tubes filled with tap water to ensure turgidity by the time the leaves were used. The test tubes were placed in racks within a sealed plastic bag containing moistened paper.

Stage 5. Selection of inoculative aphids. About three hours before the transmission tests were established, groups of 13-15 and 18-20 individuals of *M. persicae* and *U. pseudambrosiae*, respectively, were removed from the colony with a no. 00 camel's hair brush and transferred to a 9-cm Petri dish lined with moistened filter paper. The sequential order and the time at which aphid collections were completed per plate were recorded. The Petri dishes were sealed and aphids were held at room temperature.

Although the original design of the experiment called for the use of only aphids having completed the last molt, individuals of *U. pseudambrosiae* in the last one or two nymphal stages were used as well. Also, because of the small size of *M. persicae,* some immature individuals may have been overlooked and used in transmission tests. *M. persicae* has previously been reported as an efficient vector of ZYMV (Castle et al., 1992; Adlerz, 1987; Purcufull, 1984a; Lecoq et al., 1981), therefore it was included here for comparative purposes and to monitor the adequacy of the overall arena test.

Stage 6. Transmission test. A single infected leaf, contained in a plastic tube, was placed in the center of the circle of test plants as a source of virus. The test tubes were attached to a 30.5-cm garden stake to raise the infected leaves to the height of

the test seedlings. Test plants and the source leaf were in contact to allow aphids to walk from plant to plant.

Upon completion of a 2.5-h average fasting period, 10 individuals of *M. persicae* and 15 of *U. pseudambrosiae* were transferred, using a no. 00 camel's hair brush, to individual source leaves. In experiment 1, the water in the test tube was removed after 4 h with a 5-ml disposable micropipette to induce leaf wilting and to encourage any remaining aphids to leave the source of inoculum. In experiments 2 and 3, water was not removed because both aphid species were active, particularly *U. pseudambrosie,* and usually walked away from the source within a 4-6-h period. In addition, source leaves were needed for back inoculation to verify virus infection.

Five arena tests were performed for each combination of isolate-aphid vector. Two additional arenas per aphid species were included as controls. The negative and positive controls were established with a healthy leaf as a source and with a WMV-2-G2301 infected leaf, respectively. The isolate WMV-2-G2301 was transmitted by both *M. persicae* and *U. pseudambrosiae* in a previous study (Webb and Kok-Yokomi, 1993), thus it was considered a control for the ability of these species to transmit virus under the conditions of the experiment. Another type of control consisted of a pot with 4-5 watermelon plants which were not exposed to aphids. Twelve to fifteen pots of this type of control were placed along the benches close to the arena pots to check for viruliferous aphids that could have escaped from the arena tests.

During the period of aphid testing, a cast acrylic cylinder (26.5 cm) was placed onto each pot and covered with nylon organdy secured with elastic. Cylinders were removed after 24 h in experiment 1 and after 48 h in further experiments. Source leaves were collected and used for back inoculation to watermelon 'Fiesta' with the procedure already described. Insecticide was applied after removal of cylinders. Arenas were maintained in the glasshouse at 22 ± 3°C and 24-h fluorescent light for four weeks.

Upon development of symptoms, each plant was tagged and tested with enzyme-linked inmunosorbent assay (ELISA) as described by Clark and Adams (1977). In experiment 1, groups of 2-4 plants with similar symptoms were sampled and tested using the polyclonal antisera 1160 (ZYMV) and 1134 (WMV-2). In the remaining experiments, all symptomatic plants were sampled and tested with ELISA individually, except those plants from the positive control arena (WMV-2-G2301), which were sampled in groups. Nonsymptomatic plants were sampled in groups in all experiments.

Data analysis. Each experiment, consisting of 24 arena tests, was repeated three times. Percent of infected plants per arena was recorded and transformed for normality with the arc-sine of the square root of percentage values. Because the number of individuals per species (10 and 15 per arena) and the inoculation time of both ZYMV-FC3326 and ZYMV-A59F91S into sources varied within and among experiments, only statistical comparisons between isolates were done per aphid species within experiments. Data were analyzed with the procedure TTEST of SAS Institute (SAS Institute, Inc. 1988). Comparison of transmissibility between aphid species was not attempted statistically.

Results

Arena tests with *Uroleucon pseudambrosiae*. ZYMV-FC3326 was transmitted in 2 out of 50 watermelon plants in experiment 1, 4 out of 50 in experiment 2, and 0 out of 50 in experiment 3 (Table 5.3). Overall, a total of 6 out of 150 plants (i.e., 4%) were infected in 15 arena tests (Table 5.4). ZYMV-A58F91S was transmitted in 0 out of 50 plants in experiments 1 and 2, and in 15 out of 50 in experiment 3. Overall a total of 15 out of 150 plants (i.e. 10%) were infected in 15 arena tests. Plants used as negative controls remained healthy throughout the experiments as verified by visual inspection and ELISA performed on grouped samples. Only one plant in one out of three arenas

used to verify transmissibility by the *U. pseudambrosiae* clone (i.e. positive controls) was positive for WMV-2 infection.

Arena tests with *Myzus persicae*. ZYMV-FC3326 was transmitted to 18 out of 47 watermelon plants in experiment 1, 14 out of 51 in experiment 2, and in 14 out of 49 in experiment 3 (Table 5.3). Overall, a total of 46 out of 147 plants (i.e., 31.3%) were infected in 15 arena tests (Table 5.4). ZYMV-A58F91S was transmitted in 0 out of 50 plants in experiments 1, 9 out of 50 in experiments 2, and in 23 out of 50 in experiment 3. Overall a total of 32 out of 150 plants (i.e. 21.3%) were infected in 15 arena tests. Plants used as negative controls remained healthy throughout the experiments. In positive controls, WMV-2-G2301 was transmitted to 8 of 10, 7 of 10, and 1 of 10 plant in three arenas of experiments 1, 2 and 3, respectively.

Table 5.3. Proportion of watermelon plants infected per single arena test with two isolates of zucchini yellow mosaic virus (ZYMV) transmitted by two aphid species.

Experiment	[1]Isolate	[2]Proportion of infected plants in arena test: 1	2	3	4	5	Total
		Uroleucon pseudambrosiae					
1	FC3326	2 / 10	0 / 10	0 / 10	0 / 10	0 / 10	2 / 50
	A58F91S	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 50
2	FC3326	1 / 10	1 / 10	2 / 10	0 / 10	0 / 10	4 / 50
	A58F91S	0 / 10	0 / 12	0 / 10	0 / 10	0 / 10	0 / 52
3	FC3326	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 50
	A58F91S	3 / 10	3 / 10	0 / 10	6 / 10	3 / 10	15 / 50
		Myzus persicae					
1	FC3326	8 / 9	5 / 8	5 / 10	0 / 10	0 / 10	18 / 47
	A58F91S	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 50
2	FC3326	6 / 11	2 / 10	1 / 10	3 / 10	2 / 10	14 / 51
	A58F91S	1 / 10	3 / 10	1 / 10	1 / 10	3 / 10	9 / 50
3	FC3326	1 / 10	2 / 10	3 / 10	1 / 9	7 / 10	14 / 49
	A58F91S	6 / 10	3 / 10	8 / 10	1 / 10	5 / 10	23 / 50

[1]zucchini yellow mosaic virus isolates: FC3326 = field isolate collected from a single watermelon plant on 2 May, spring 1994; A58F91S = isolate collected from squash in September 1991 and maintained in greenhouse by mechanical passage.
[2]Five arena tests performed per experiment for each combination of isolate and aphid species. Results given as proportion in which: numerator = number of infected plants, denominator = total number of watermelon plants tested.

Table 5.4. Proportion of watermelon plants infected with two isolates of zucchini yellow mosaic virus (ZYMV) transmitted by apterae of two aphid species in arena tests.

Specie	[1]Isolate	[2]Exp 1	Exp 2	Exp 3	Total
Uroleucon pseudambrosiae	FC3326	2 / 50	4 / 50	0 / 50	6 / 150
	A58F91S	0 / 50	0 / 50	15 / 50	15 / 150
Myzus persicae	FC3326	18 / 47	14 / 51	14 / 49	46 / 147
	A58F91S	0 / 50	9 / 50	23 / 50	32 / 150

[1]Zucchini yellow mosaic virus isolates: FC3326 = field isolate collected from a single watermelon plant on 2 May, spring 1994; A58F91S = isolate collected from squash in September 1991 and maintained in greenhouse by mecanical passage.
[2]Five arena tests performed per experiment for each combination of isolate and aphid species. Results given as proportion in which: numerator = number of infected plants, denominator = total number of watermelon plants tested. Most arena test consisted of 10 plants (see Table 5.3).

Variability of transmission results. Variability of transmission results with *U. pseudambrosiae* among arenas using the same virus isolate within each experiment was in the range of 0% (0 / 10) to 20% (2 / 10) for ZYMV-3326 and of 0% (0 / 10) to 60% (6 / 10) for ZYMV-A58F91S. With *M. persicae*, the variability was in the range of 0% (0 / 10) to 88.9% (8 / 9) for ZYMV-3326 and of 0% 0 / 10) to 80% (8 / 10) for ZYMV-A58F91S (Table 5.3).

Variability of sources of inoculum. Symptoms induced by the two isolates of ZYMV differed in their time of expression after inoculation as well as in the range of symptoms observed. Symptoms induced by ZYMV-A58F91S were more uniform than those induced by ZYMV-FC3326.

Although both ZYMV-FC3326 and ZYMV-A58F91S induced severe necrosis on test plants, symptoms induced by ZYMV-A58F91S appeared at least one week earlier. Because of that, this isolate eventually killed some young seedlings or made the host tissue unavailable for use in transmission tests due to either severe stunting or apparent host recuperation. To overcome this difference in time of expression and perhaps in virus titer, in experiments 2 and 3, older seedlings having one or two true leaves were used as sources for ZYMV-A58F91S.

Thus, sources for ZYMV-FC3326 and ZYMV-A58F91S differed in their time of infection at their use in transmission tests, i.e., respectively, 26 vs. 9 days in experiment 2, and 31 vs. 15 days in experiment 3. For experiment 1, time of infection for all virus isolates was 32 days.

Discussion

The ability of *U. pseudambrosiae* to transmit two Florida isolates of ZYMV was demonstrated in this study. Adlerz (1987) showed experimentally that one out of nine *Uroleucon* sp. collected from pan and suction traps was able to transmit ZYMV in Central Florida. Although only two species in the genus *Uroleucon*, i.e., *U. sonchellus* (Monell) and *U. pseudambrosiae* have been trapped at Leesburg since 1992 (Table 4.3 in Chapter 4), at least one other species, *U. ambrosiae* (Thomas), has been caught in Florida (Webb and Kok-Yokomi, 1993). Therefore it is not certain that *U. pseudambrosiae* may have been the species found transmitting ZYMV by Adlerz (Adlerz, 1987).

In addition to the confirmation in this study of *U. pseudambrosiae* as a vector of ZYMV, at least three other viruses, all of them of the genus *Potyvirus,* have been reported as being transmitted by this aphid: WMV-2 and papaya ringspot virus type watermelon (Webb and Kok-Yokomi, 1993) and bean common mosaic virus (Zettler, 1967). Overall, at least three other species of *Uroleucon* have been found transmitting different plant viruses: *U. formosanum* (Takahashi) and *U. gobonis* (Matsumura) vectoring WMV-2 (Yamamoto et al., 1982, Table 2.2 in Chapter 2); *U. ambrosiae* (Thomas) transmitting ZYMV, WMV-2, and maize dwarf mosaic virus (Orosco-Santos et al., 1994; De Sa and Kitajima, 1991; Knoke et al., 1977; Table 2.2 and 2.4); and *U. sonchi* as a vector of cucumber mosaic virus and potato virus Y (Raccah et al., 1985).

The biological confirmation of *U. pseudambrosiae* as a vector of ZYMV supports the previous identification of this species, through statistical methods, as a major vector in secondary viral dispersion under field conditions (Chapter 4). These results suggest that, although results derived from correlative studies should be interpreted cautiously and be biologically verified, statistical approaches can be used to determine putative important vectors under certain conditions.

M. persicae has previously been reported as a vector of ZYMV in field live assays and with single or multiple aphid transfers under controlled conditions of both acquisition access and inoculation access (Castle et al., 1992; Adlerz, 1987; Purcifull, 1984a; Lecoq et al., 1981). The transmission rate of ZYMV by *M. persicae* under controlled conditions has been in the range of 20% to 80% (Castle et al., 1992; Adlerz, 1987; Purcifull, 1984a; Lecoq et al., 1981). In field live assays, however, lower values of transmission rates have been reported, i.e., 0.006 and 0.02 (Castle et al., 1992; Adlerz, 1987). In this study, the proportion of infected plants was 31.3% and 21.3% for the isolates ZYMV-FC3326 and ZYMV-A58F91S, respectively.

Because the proportion of infected plants in arena tests does not estimate transmission rates directly, the transmissibility results with *M. persicae* can not be compared with previous studies. Also, comparison of abilities of transmission of *M. persicae* and *U. pseudambrosiae* in this study are not fully valid because of the different number of individuals used per species (i.e., 10 and 15, respectively). Fifteen individuals of *U. pseudambrosiae* were selected more or less arbitrarily, based on an exploratory test and with the knowledge that 10 individuals did not transmit ZYMV in a previous attempt (Webb and Kok-Yokomi, 1993).

Whereas the estimation of transmission rates are obtained directly in controlled tests with the single aphid transfer method (Swallow, 1985), and a method has been proposed to derive it when a multiple individuals transfer method is used (Swallow, 1985; Gibbs and Gower, 1960), no procedure has been proposed to estimate

transmission rates with arena tests. The convenience of such procedures could be valuable for appropriate comparisons of situations such as those outlined in this study and for some specific epidemiological applications such as the determination of the parameters '*infectivity index*' or '*vector intensity*' essential in simulation models (Plumb et al., 1986; Ruesink and Irwin, 1986; Irwin and Ruesink, 1986).

Using the percent of infected plants, as it has been done in arena tests (Webb and Kok-Yokomi, 1993; Summers et al., 1990; Halbert et al., 1994), and keeping in mind the questionable validity of such a comparison because of the different number of individuals tested, the transmissibility of ZYMV-FC3326 and ZYMV-A58F91S by *U. pseudambrosiae* appears to be relatively lower than that of *M. persicae* (4% and 10% vs. 31.3% and 21.3%, respectively). These results can not be used directly, however, to estimate the relative importance of these vectors under field conditions even though the arena test, an approach that estimates '*vector propensity*' under more natural conditions (Irwin and Ruesink, 1986) was used.

The apparent lower transmission ability of *U. pseudambrosiae* can be compensated for by high field population densities. For example, a total of 137 and 1390 individuals of this species were caught in green tile traps in Leesburg, FL in 1993 and 1994, respectively. In contrast, only 15 and 113 individuals of *M. persicae* were, trapped, respectively, in those years. Thus, it is the integration of transmission ability and population density as well as the seasonal distribution of the population which finally determines the epidemiological importance of a vector (Plumb et al., 1986; Irwin and Ruesink, 1986).

Comparison of results of the arenas established with the isolate WMV-2-G2301 (positive controls) with a previous study in which the aphid clones and the WMV-2 isolate used in this study were originally used (Webb and Kok-Yokomi, 1993), indicates that the clones of *U. pseudambrosiae* and *M. persicae,* in general, maintained their

original ability to transmit WMV-2-G2301, even though the proportion of plants infected was apparently lower.

The reduced number of arenas as well as some differences in experimental conditions may explain the apparently lower transmissibility of WMV-2-G2301 in this research. Fifteen arenas per species were used by Webb and Kok-Yokomi (1993) whereas only three were used in this study. Such low numbers could reduce reliability of arena results when testing aphids with relatively low efficiency of transmission as appears to be the case with *U. pseudambrosiae* (Webb and Kok-Yokomi, 1993) and perhaps with *Diuraphis noxia* Kurdjumov transmitting bean common mosaic virus (Summers et al., 1990; Halbert et al., 1994).

The average percent of infected plants with WMV-2-G2301 by *U. pseudambrosiae* in this study was 3.33% ± 5.77 SD, in contrast to a 23% ± 12 SD found in the previous study (Webb and Kok-Yokomi, 1993). With *M. persicae*, however, results were statistically similar even though transmissibility was slightly lower in this study, i.e., mean = 53.3 % ± SD 37.9, vs. mean = 61% ± SD 19 (Webb and Kok-Yokomi, 1993).

Although it is not clear that the degree of repeatability of transmission values with WMV-2 can be used to indicate the validity of results of transmissibility with other virus isolates, i.e., ZYMV-FC3326 or A58F91S, provided that the same clone of aphid is used, it could provide clues to evaluate the overall conditions of the experiments. In addition, the inclusion of a system previously tested as a control or standard in a new test, could allow the generation of valuable conclusions in comparative studies (Halbert et al., 1994).

In general, great variability has been observed in arena results, perhaps due to the free behavior of the vector in acquiring and inoculating the virus (Webb and Kok-Yokomi, 1993; Summers et al., 1990). Although a behavioral component is inherent to arena tests (Irwin and Ruesink, 1986), experimental conditions may have an effect on

reliability of results in a similar way as they do in controlled tests (Labonne et al., 1992; Fereres et al.,1992; Sylvester, 1954; Watson, 1936).

In this study, large variability of results was observed within and between experiments (Table 5.3), perhaps due to a great extent to the quality of the inoculum. When the age of the inoculum was in the range of 26 to 32 days (i.e., ZYMV-FC3326), total standard deviation of the mean was smaller for both aphid species than when the age of inoculum was in the range of 9 to 32 days (i.e., ZYMV-A58F91S): 4% ± 4 SD and 31.44% ± 5.97 SD vs. 10% ± 17.32 SD and 21.3% ± 23.18 SD. Higher transmission of ZYMV-A58F91S by both aphid species was observed when the age of inoculum was 15 days.

Thus, the apparent variability of the virus titer in the sources appeared to be an important cause of variation in this research and in other studies in which the transmissibility under controlled conditions of ZYMV by *M. persicae* dropped as the inoculum aged (Castle, 1992). In Castle's study, transmissibility of WMV-2 by the same vector did not drop in the range of age studied (from 1 to 4 wk) (Castle, 1992).

Because of the variability observed, no solid conclusions can be established regarding the suggested idea that ZYMV-FC3326 could be an isolate similar to ZYMV-A58F91S (Chapter 3), even though statistical analyses were performed per experiment in an attempt to reduce variability. The percentages of infected plants with ZYMV-FC3326 were significantly higher in the first two experiments, but the opposite result was obtained in the last experiment.

ZYMV-A58F91S has been passaged mechanically, a condition that may result in loss of transmissibility in some ZYMV isolates (Lecoq and Purcifull, 1992) and in other potyviruses (Koike, 1979; Simons, 1976; Evans and Zettler, 1970; Swenson, 1957). Because ZYMV-A58F91S was transmissible, its probability of success at the field level could be potentially high and thus its similarity to ZYMV-FC3326 can not be ruled out.

On the other hand, the longer time needed for symptom expression in sources after mechanical inoculation and in arenas as a result of vector transmission, as well as in the broader range of symptoms induced by ZYMV-FC3326, suggest, that some biological differences do exist between these two isolates. However, these apparent biological differences are not conclusive enough to define a nonidentity relationship between the two isolates. Consequently, the idea that spring virus epidemics in experimental plots at Leesburg, FL could have originated from an accidental release of a greenhouse-maintained isolate of ZYMV (G. W. Elmstrom, personal communication) can not be ascertained solely on results of this study.

CHAPTER 6
SUMMARY AND CONCLUSIONS

Spring virus epidemics in watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] were described in North Central Florida. Characterization of virus disease incidence as it relates to temporal spread, spatial patterns, yield loss, and aphid population dynamics was addressed in this study.

Studies were conducted with experimental watermelon plots established in the spring of 1993 and 1994 at Leesburg, FL and extensively monitored for signs of virus infection through the season. Identity of infecting viruses was assessed with enzyme-linked immunosorbent assays (ELISA) and sodium dodecyl sulfate (SDS)-immunodiffusion tests using antisera to the common viruses found in cucurbit crops in Florida. Effect of virus infection on fruit production and fruit sugar content was determined based on harvesting of single plants. Information regarding abundance and composition of aphid species was obtained by daily trapping, and transmission tests were set up with a predominant aphid species not previously reported as a vector of a common potyvirus found in 1994.

Standard epidemiological analyses as well as new analytical approaches were used at different stages in this research.

Temporal Spread of Viral Disease

Progress of spring virus epidemics followed symmetrically sigmoid curves. Epidemics were best described by the logistic model with average apparent infection rates ranging from 0.193 to 0.225 units day^{-1} in 1993 and from 0.320 to 0.409 units day^{-1} in 1994. The r-square values associated with this model were higher than 0.93 ($p \leq 0.05$) for two years. In general, epidemic rates were statistically different between but not within years ($t_{est} \geq t$, $P = 0.05$). The first infected plants were detected on 30 April

(1993) and 25 April (1994), 40 and 41 days after planting, respectively. In 1993, 93-98% final disease incidences were reached at 91 to 97 days after planting. In 1994, 100% incidence was recorded at 69 to 76 days after planting.

In 1993, watermelon mosaic virus (WMV-2) was detected with ELISA in 97% of 294 systematically selected plants on 7 June. In 1994, WMV-2 alone or mixed with ZYMV was detected with ELISA in only 12% of 298 plants in one plot on 26 May, whereas ZYMV was detected in 80% of the 298 plants . ZYMV and WMV-2 were also detected with SDS-immunodiffusion on 28 April in the first plants found with symptoms of viral infection. WMV-2 but not ZYMV, was detected in four commercial fields randomly sampled on 18 and 21 May, 1994.

Papaya ringspot virus type watermelon (PRSV-W), tested in extensive sampling with ELISA, and cucumber mosaic, squash mosaic, trichosanthes virus, and 2932 (an uncharacterized potyvirus), tested in restricted sampling with SDS-immunodiffusion were not detected in either year.

Spatial Patterns of Viral Disease Spread

Disease incidence gradients toward a local, experimental field containing infected watermelon plants were best described with a version equation of the diffusion model (nonstationary wave model). Fairly clear wave shapes of dispersion were observed early in the season, on 2 May in 1994. Within six days, disease gradients flattened, indicating high secondary dispersion within the field. Individual sources within the field, in the range of 40-200 meters, could have contributed to the dispersal gradients found in this study.

A parameter of diffusivity of inoculum from the source (D_0) and a parameter of gradient shape (m) were estimated at different dates and levels of disease incidence. The parameter D_0, in general, decreased with time from 0.012 to 0.010 in one plot and

from 0.011 to 0.004 in a second plot. Statistically, only in one case (D_0 = 0.018 -5 May- vs. D_0= 0.004 -8 May-) were the diffusion parameters significantly different, as indicated by a non-overlapping confidence interval at P=0.05 (C.I. 95%). The parameter m also decreased with time from values higher than 1 to values close to or lower than 1. No statistical differences were detected among values of this parameter.

The estimated diffusion parameter of disease gradients observed on 2 May in the two plots studied had similar values (D_0= 0.012 and 0.011). Differences in this parameter value, although not statistically significant, were higher on the following dates although comparisons may not be appropriate because of strong discrepancies in model goodness-of-fit on those dates. The shape values of disease gradients differed on most dates studied.

Yield Loss Modeling

Average yield by single plants or by all plants infected at the same time were well correlated with the period in days in which plants remained healthy. The highest r^2-value was achieved with a model without intercept. Yield increase by plant was directly proportional to the period in which plants remained healthy with a rate of 0.146 kg day^{-1} (1993) and 0.140 kg day^{-1} (1994). In 1993, the average fruit sugar content of plants that remained healthy the same period of time was not correlated with time of infection (r^2 = 0.046, P > F = 0.50). Significant correlation of sugar content was found, however, in 1994 (r^2 = 0.818 P > F = 0.05).

Aphid Population Dynamics and Virus Disease Incidence

Abundance and aphid species composition. From a total of 37 aphid species collected in 1993 and 1994, 16 species corresponded to known vectors of WMV-2, 9 of ZYMV, and 13 of PRSV-W. The most abundant species trapped in the two seasons

which were known to be vectors of at least one virus were: *Aphis craccivora* Koch, *A. gossypii* Glover, *A. spiraecola (=A. citricola)* Patch, and *Myzus persicae* (Sulzer) of WMV-2, ZYMV, and PRSV-W; *Aphis middletonii* (Thomas) of WMV-2 and ZYMV; *Uroleucon pseudambrosiae* (Olive) of WMV-2 and PRSV-W, and *Lipaphis erysimi* (Kaltenbach) of ZYMV and PRSV-W. One aphid species, known to be a vector of at least one of these viruses, was detected for the first time in Florida: *Acyrthosiphon kondoi* Shinji, the blue alfalfa aphid.

The abundance of these species per season was as follows: in 1993, *Uroleucon pseudambrosiae* (137 out of 462 total aphid vectors), *Aphis middletonii* (137 out of 462), and *A. gossypii* (119 out of 462) were the most common aphid vectors caught in green tile water traps. The highest peaks of these species were 14 May, 25 April and 5 May, respectively. *A. spiraecola, A. craccivora* and *Myzus persicae* were less frequently captured.

In 1994, the aphids *U. pseudambrosiae* (1390 out of 1803 total aphid vectors), *M. persicae* (113 out of 1803), and *A. middletonii* (97 out of 1803), were the most common aphid vectors caught. The highest peaks of these vectors were 15 April and 4 May (*U. pseudambrosiae)*, 25 April (*M. persicae*), and 20 April (*A. middletonii*).

Statistical correlation of aphid species with disease incidence. Principal component analysis and varimax rotated biplots of the major principal components were integrated with multiple regression analysis to examine the association of the number of aphid vectors (as independent variables) with changes in disease incidence.

The best model to describe the association of vector density with disease change in 1993 was: $\hat{y} = 0.09Up + 0.10\,Am + 0.11\,Ag$, in which $\hat{y}$ represents the prediction in change of disease incidence as $\sum (Y_t - Y_{t,1})$ at any given time; Up is the number of *Uroleucon pseudambrosiae* individuals trapped at the 21st day before the disease assessment and transformed as PC-scores; Am (*Aphis middletonii*) and Ag (*A. gossypii*) are PC-scores of counts of individuals in the 35th and 32nd day before the

disease assessment was made. The precision of estimates with this model was 82% ($p=0.0005$), all regression parameters were significant ($p \leq 0.021$), and the Mallows's C_p value was close to the number of parameters in the model ($C_p = 2.38$).

The best explanatory model in 1994 was: $\hat{y} = 0.14Up + 0.12\ (Am)(As)$, in which $\hat{y}$ represents the prediction of change in disease incidence as $\Sigma\ (Y_t - Y_{t-1})$ at any given time; *Up* is the number of *U. pseudambrosiae* individuals trapped at the 12th day before the disease assessment and transformed as PC-scores; *Am* (*Aphis middletonii*) and *As* (*A. spiraecola*) are PC-scores of counts of individuals at the 22nd and 24th day before the disease assessment was made. The precision of estimates with this model was 94% ($p=0.0001$), all regression parameters were significant ($p \leq 0.003$), and the Mallows's C_p value was close to the number of parameters in the model ($C_p = 1$).

Arena test. Because *U. pseudambrosiae* appeared to play a major role in the secondary spread of both WMV-2 and ZYMV as the statistical models and the seasonal distribution of its population suggested, and because no previous reports indicated the transmission of ZYMV by this aphid, arena tests were conducted in this study. ZYMV-FC3326, an isolate collected from one experimental plot in 1994, was transmitted to 6 out of 150 plants tested in a total of 15 arenas. ZYMV-A58F91S, an isolate collected in 1991, was transmitted to 15 out of 150 plants. The known vector *Myzus persicae*, included in these tests as a standard, transmitted the virus to 46 out of 147 plants (ZYMV-FC3326) and to 32 out of 150 plants (ZYMV-A58F91S). The different level of transmissibility of these two ZYMV isolates was not conclusively confirmed because of the high variability of arena results.

Conclusions

- Watermelon mosaic virus type 2 (WMV-2) continues to be the most common virus affecting spring watermelon production in Central Florida. Zucchini yellow mosaic

virus (ZYMV) continues to be a potential threat to the watermelon industry when inoculum sources are abundant. Fortunately, this situation has not occurred very often in North Central Florida.

- WMV-2 was the only virus associated with spring epidemics in 1993. ZYMV was most prevalent in 1994. Higher epidemic rates, earlier onset, shorter epidemic duration, and shorter time to reach 50% disease incidence were found in 1994.
- An estimated fruit yield lost in cv. "Fiesta" of 26% due to WMV-2 and 31% due mostly to ZYMV were registered in 1993 and 1994, respectively. Significant reduction of fruit sugar content below 11°Brix, the standard for the cultivar, was only recorded in 1994.
- At least 16 and 9 aphid species known to be vectors of WMV-2 and ZYMV, respectively, were detected in the spring season in Central Florida. Of these species, *Uroleucon pseudambrosiae* (Olive) was proved to be a vector of ZYMV and *Acyrthosiphon kondoi* Shinji was detected for the first time in Florida.
- The species *A. middletonii* and *U. pseudambrosiae* were identified as important vectors to describe changes of disease incidence induced by WMV-2 in 1993 and mostly ZYMV in 1994 in spring watermelon in Central Florida.
- The species *A. middletonii* appears to play its major role in primary virus dispersion from local or distant infected sources. *U. peudambrosiae* appears to be involved in secondary virus dispersion either within fields or to adjacent fields. The highest flight activity of these species was detected from 20 to 25 April, and from 4 to 14 May, respectively.
- Two major components appear to be essential to the development of spring virus epidemics in watermelon in Central Florida: a) abundance of sources of inoculum particularly in the vicinity of the field, and b) high population densities of major vectors *Uroleucon pseudambrosiae* and *Aphis middletonii* and of minor vectors *A. spiraecola* and *A. gossypii* under some specific conditions.

APPENDIX 1
PHOTOGRAPHS OF COMMON APHID VECTORS

Morphological descriptions of the aphid species *Aphis sp* (possibly *Aphis fabae solanella*), *A. gossypii*, *A. middletonii*, *A. spiraecola*, *A. craccivora*, *Myzus persicae*, *Uroleucon pseudambrosiae*, and *U. ambrosiae* are included in this appendix. These aphid species are worldwide vectors of WMV-2, PRSV-W and/or ZYMV in watermelon (*Citrullus lanatus*). With the exception of *U. ambrosiae*, which was not caught in this study, these aphids were commonly trapped in green tile water traps in experimental plots of watermelon at Leesburg, Florida.

U. ambrosiae was included in this section because of morphological similarities with *U. pseudambrosiae*. Two mounted specimens of *U. ambrosiae* were obtained from Division of Plant Industry (DPI) of Gainesville, Florida for comparative purposes. These two aphids were collected from the weeds *Coreopsis leavenworthii* and *Iva imbricata* in Port St. Lucie, FL and Ponte Vedra, FL, respectively.

The aphids photographed were mounted by M. L. Kok-Yokomi and identified by S. E. Webb. Identifications were confirmed by S. E. Halbert. Morphological descriptions were based on several identification keys developed for alate aphids trapped with suction, wind and/or yellow traps (Basky, 1993; Smith et al., 1992; Maedler and Ghosh, 1969). Other keys for both alates and apterae were also used to provide information about host, color of aphids, and other characteristics (Brown, 1989; Holman, 1974).

Full body pictures of aphids were taken with a stereoscopic microscope Wild M3Z®, Heerbrugg Switzerland with an T-Q / F0I-1 illumination system, Techni-Quip

Corp. Hollywood, Cal. The best contrast for this type of picture was obtained with 100 ASA 135 mm AGFAPAN APX 100 film and orange paper used as slide background.

Partial body pictures of aphids were obtained with a Leitz Laborlux S® light microscope attached to a Wild Leitz MPS 46 Photo automat® system, Leitz, Wetzlar, Germany. Best results were obtained with a 125 ASA 135 mm ILFORD FP4 DX PLUS film.

General Characteristics of Aphididae

Aphididae is the biggest family of the order Homoptera and contains most of the aphid species known to be vectors of plant viruses on a worldwide basis. Characteristics of the family include: antennae 0.3 (apterous) or more than 0.5 (alate) times the body length, 4-6 segmented with two primary rhinaria in the last two antennal segments (Plate 4), secondary rhinaria rounded or oval (Plate 4), siphunculus usually well developed (Plate 3-10), cauda knobbed (subfamilies Chaitophorinae and Drepanosiphinae) or varying greatly in shape, usually distinctly elongated. If the cauda is not knobbed, then the siphunculus is a hairy cone or a mere rim or pore, and numerous setae uniform cover the body (subfamily Lachninae), or siphunculi are elongated, usually more than 2 times width and with less numerous setae (subfamily Aphidinae) (Plate 3-10). If siphunculi are elongated, abdominal segments I and VII have marginal tubercles (tribe Aphidini, subtribe Aphidina, subtribe Rhopalosiphina) (Plate 4, Plate 6) or do not (tribe Macrosiphini) (Plate 9) (Holman, 1974, Medler and Ghosh, 1969).

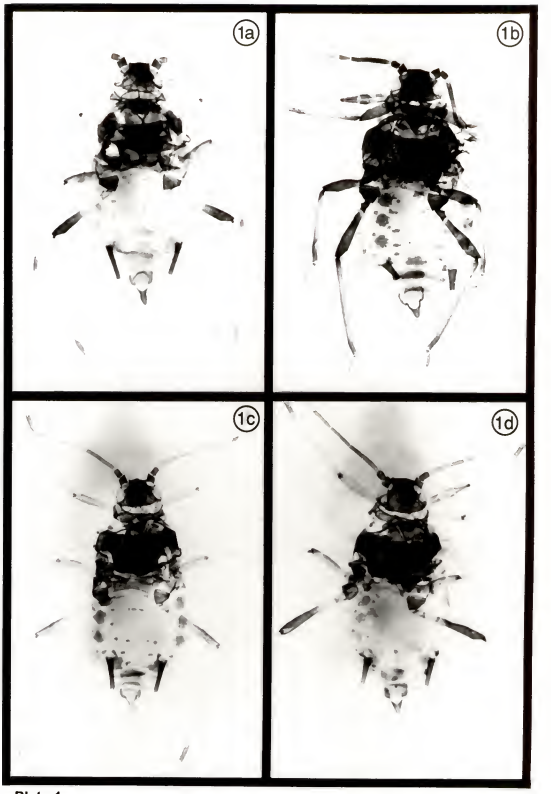


Plate 1.

1a. *Aphis craccivora* Koch
1b. *Aphis middletonii* Thomas
1c. *Aphis gossypii* Glover
1d. *Aphis* sp.

Plate 2.

2a. *Aphis spiraecola* Patch
2b. *Myzus persicae* (Sulzer)
2c. *Uroleucon pseudambrosiae* (Olive)
2d. *Uroleucon ambrosiae* (Thomas)

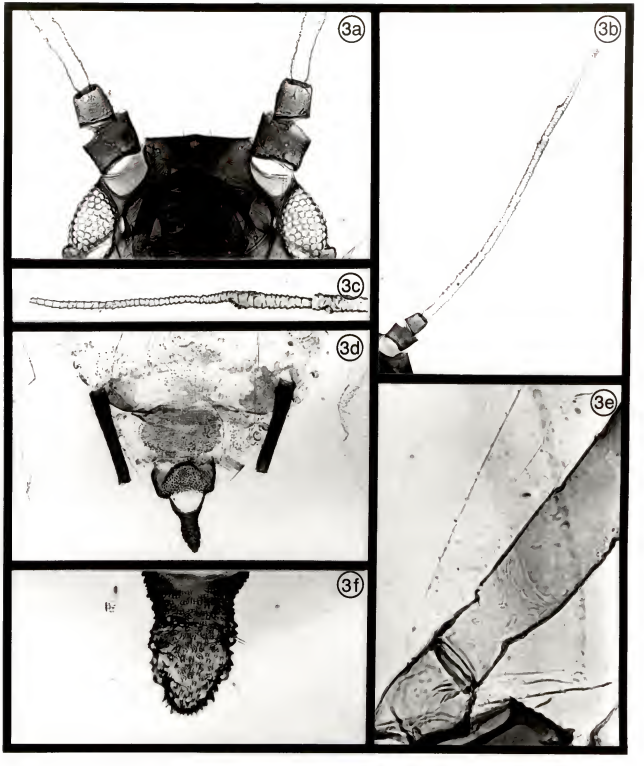


Plate 3. *Aphis craccivora* Koch. 3a. Head with weakly developed frontal tubercles. 3b. Antenna 6-segmented, A.s. III with 3-8 secondary rhinaria irregular in size. 3c. Processus terminalis 1.9-2.7 times base of last segment. 3d. Dorsal abdomen with extensive black patch centered on tergites 4-5. 3e. Femoral setae 0.5-0.7 times width of femora. 3f. Cauda with 6-7 setae, rarely 4-5.

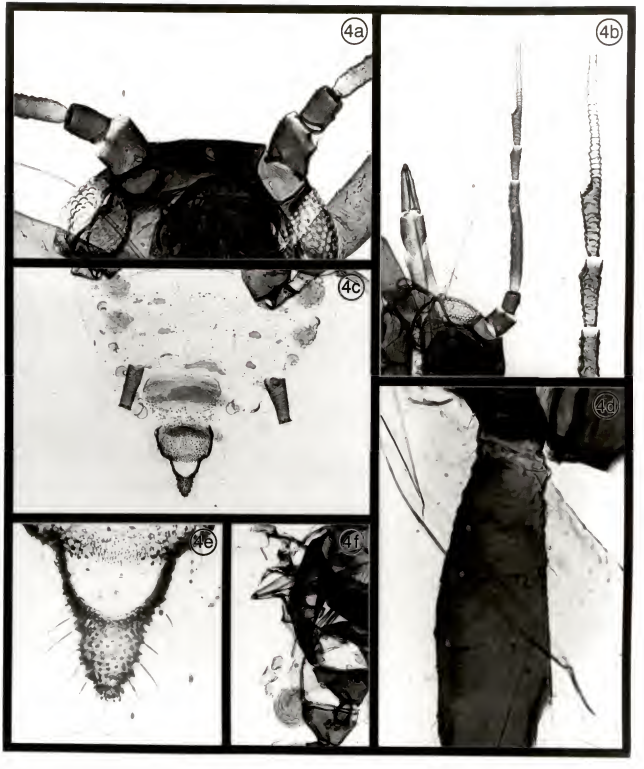


Plate 4. *Aphis middletonii* Thomas. 4a. Head with weakly developed frontal tubercles. 4b. Antenna 5-6 segmented, processus terminalis 1.5-1.9 times base of last segment. 4c. Dorsal sclerotized bars only on posterior segments, marginal sclerites conspicuous. 4d. Femoral setae shorter than width of femora. 4e. Cauda with more than 10 setae. 4f. Lateral abdominal tubercles on segments I and VII conspicuous (Plate 4c).

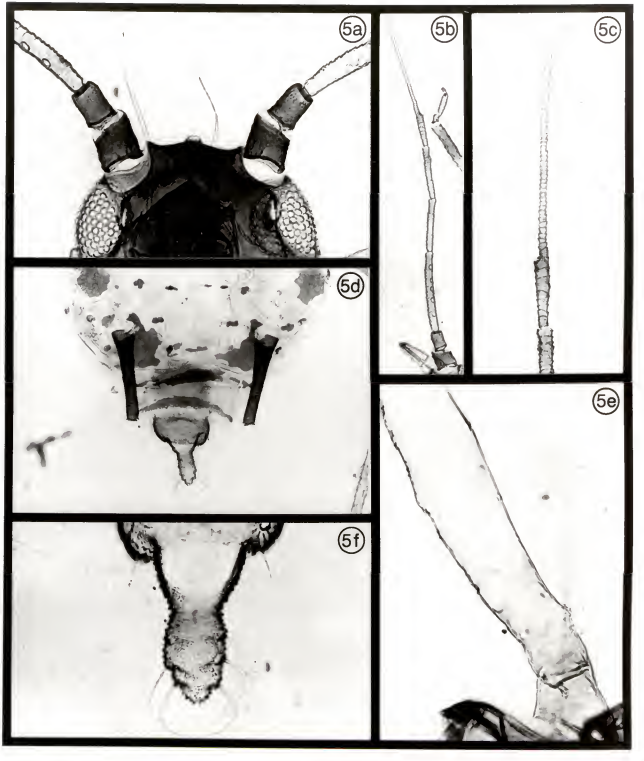


Plate 5. *Aphis gossypii* Glover. 5a. Head with weakly developed frontal tubercles. 5b. Antenna 6-segmented, occasionally 5 segments in summer specimens. A.s. III with 3-9 secondary rhinaria in a row, regular in size, conspicuous. A.s. III > A.s. IV. 5c. Processus terminalis 2-3 times base of last segment. 5d. Often with sclerotized dashes on dorsum (VI, VII, and VIII), postsiphuncular sclererite present. 5e. Femoral setae 0.5-0.7 times width of femora. 5f. Cauda with 4 curved setae, rarely 5-7, paler than siphunculus.

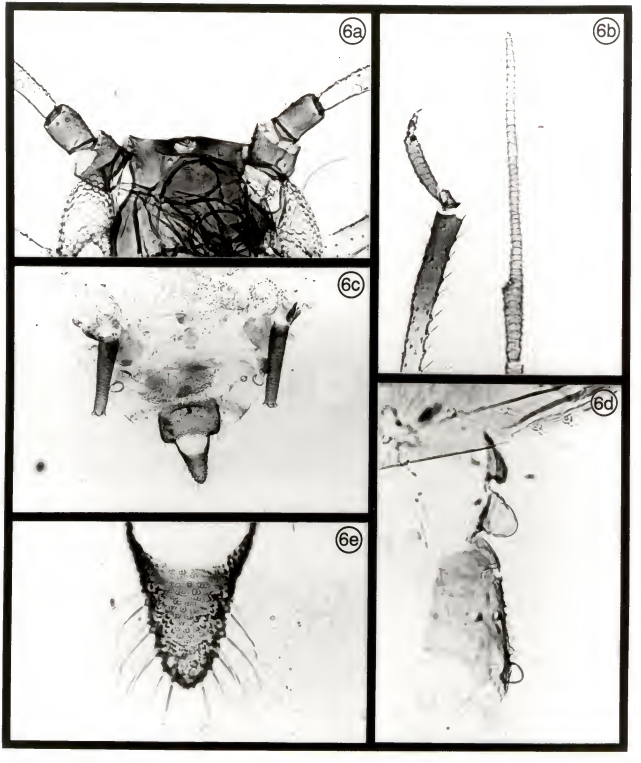


Plate 6. *Aphis* sp. 6a. Head with weakly developed frontal tubercles. 6b. Processus terminalis 2-3 times base of last segment. Antenna 6-segmented, A.s. III with scattered secondary rhinaria (Plate 1d). 6c. Sclerotized bars on posterior segments of dorsum. Postsiphuncular sclererite conspicuous. 6d. Femoral setae 0.5-0.7 times width of femora. Lateral abdominal tubercles on segments I and VII (Plate 6c). 6e. Cauda with 12 or more setae.

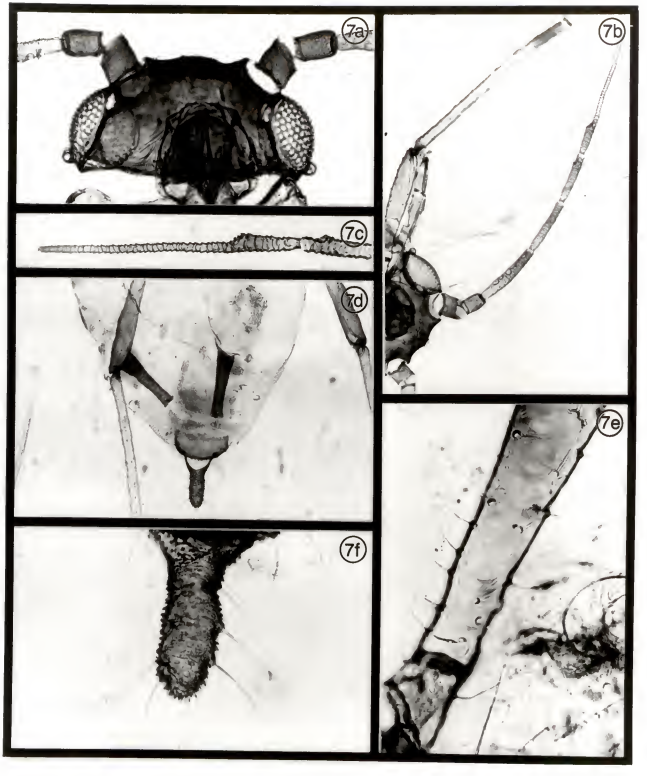


Plate 7. *Aphis spiraecola* Patch. 7a. Head with weakly developed frontal tubercles. 7b. Antenna 6-segmented, sometimes 5 in summer specimens. A.s. III with 4-10 secondary rhinaria, inconspicuous. A.s. III and IV similar in length. 7c. Processus terminalis 2-3 times base of last segment. 7d. Dorsal bars absent, postsiphuncular sclererite not conspicuous. 7e. Femoral setae fine, about width of femora. 7f. Cauda with 6-12 fine setae, constricted at base. Cauda as dark as siphunculus.

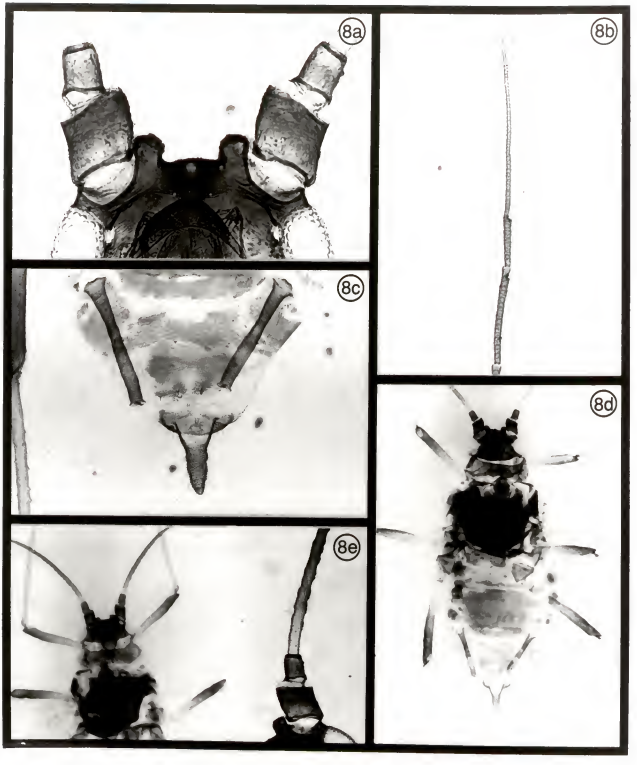


Plate 8. *Myzus persicae* (Sulzer). 8a. Head with converging frontal tubercles. 8b. Processus terminalis > 3 times base of last segment. 8c. Siphunculi slightly swollen on the inner surface, two times the length of cauda. 8d. Large dorsal sclerotized patch, with a window. 8e. Antennal segment III with 7-16 secondary rhinaria in a single row.

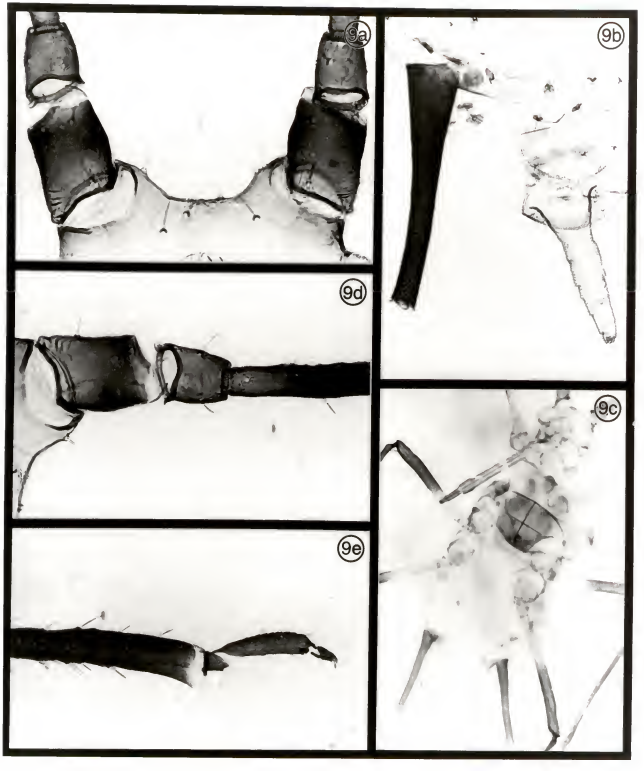


Plate 9. *Uroleucon ambrosiae* (Thomas). 9a. Head with diverging frontal tubercles. 9b. Siphunculi with distal reticulations, dark, longer than cauda. Cauda, pale with 13-23 setae. 9c. Lateral tubercles absent. 9d. Length of antennal segment I much greater than length of hind tarsal segment II (Plate 9e).

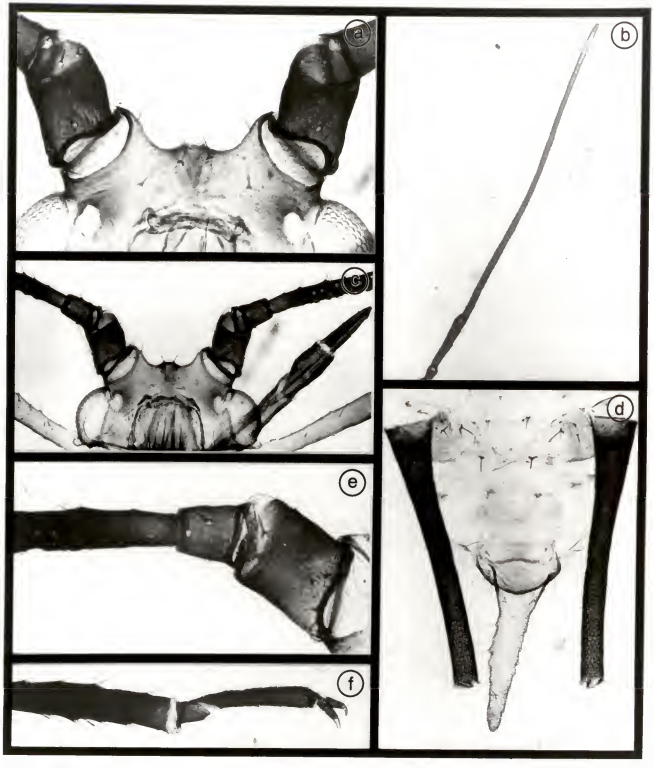


Plate 10. *Uroleucon pseudambrosiae* (Olive). 10a. Head with diverging frontal tubercles. 10b. Processus terminalis at least 5 times base of last segment. 10c. Rostrum dark dusky. 10d. Siphunculi with distal reticulations, dark, longer than cauda. Cauda, pale with 13-27 setae. Marginal sclerites dusky, antisiphuncular sclerites dusky and broken, postsiphuncular sclerites dusky and entire, lateral tubercles absent. 10e. Length of antennal segment I equal or shorter than length of hind tarsal segment II (Plate 10f).

APPENDIX 2
SAS® PROGRAMS

SAS® (Statistical Analysis System) programs used to perform specific analysis of data sets presented and discussed in Chapters 3 to 5. Programs included in this appendix are in the original format submitted to SAS 6.08 for Microsoft Windows 3.11 running on an IBM-compatible Personal System with a 66MHz 486DX2 microprocessor. Only one program was included when two series of data, i.e., 1993 and 1994, were analyzed with a similar program. The output of these programs were omitted due to space constraints.

Logistic, Gompertz, and Monomolecular /Linear models

```
*------------------------------------------------------------*
| SAS program to estimate the apparent infection rate with   |
| the logistic,Gompertz,and monomolecular linearized models. |
| Data 1994. Leesburg,Fl.  File name: Inpepi94.sas this file |
| is similar to inputepi.sas (:\wm93sas\) for data the 1993  |
*------------------------------------------------------------*;

data waterm94;
   do plot=1 to 4;
      format pdate date7.;
      format idate date7.;
       if plot=1 then do;
              pdate='15mar94'd; idate='25Apr94'd; plants=298; max=9; end; /*
idate=infection date */
       else if plot=2 then do;
         pdate='15mar94'd; idate='25Apr94'd; plants=299; max=9; end;
       else if plot=3 then do;
         pdate='15mar94'd; idate='02may94'd; plants=299; max=9; end;
       else if plot=4 then do;
         pdate='15mar94'd; idate='28Apr94'd; plants=299; max=11; end;
    DO c=1 to max;
     INFILE 'A:\wm94sas\epid.dat';
      INPUT DATE DATE7. I;     /* I=number of new infected plants */
         FORMAT DATE DATE7.;
         DAY=DATE - IDATE;
         DAY_plan=DATE - PDATE;
         inc=I/plants;                  /* Inc = % of I */
         if date=idate then ia=0;
          IA = IA +(I/plants);          /* IA= % accumulate incidence */
          if IA>=1 THEN  IA=0.99;
          IF IA=0  THEN  IA=0.001;
            L=LOG(IA/(1-IA));
            G=LOG(1/LOG(1/IA));
            M=LOG(1/(1-IA));
```

```
        OUTPUT;
    END;
  END;
proc print;  TITLE1 'VIRUSES EPIDEMICS ON WATERMELON, LEESBURG 1994';
var plot day_plan Inc IA;
run;
proc sort;by plot;
proc plot;by plot; plot IA*day='&' Inc*day='*'/overlay vpos=15 hpos=30;
title 'Plot of accumulate and nonaccumulated infected plants, Leesburg FL data
1994';
run;

proc sort; by plot;
proc glm; by plot; model L G M = day;
output out=plotdata  residuals=RL RG RM predicted=PL PG PM;
title   'Proc GLM / logistic, Gompertz, and monomolecular models';
run;
proc sort; by plot;
proc plot data=plotdata; by plot;
     plot PL*RL PG*RG PM*RM /vref=0 vpos=15 hpos=30;
title 'Evaluation of epidemiological models to WMV2-epid Leesburg94';

run;
```

Yield Loss Modeling: Data Set 1993

```
 *-------------------------------------------------*
 |  Watermelon yield 1993, Leesburg,FL             |
 |  Yield Loss Modeling,                           |
 |  prod93\prodinp.sas                             |
 *-------------------------------------------------*;

data prod93;
infile 'a:\prod93\prosas93.dat';
  input obs id $ Weigh1 sugar;
 /* WP-asci format problem to read with infile/so direct print from data and then use
infile */
  drop obs;
  Weigh=Weigh1*0.45359;       /* conversion of pounds to kilograms */
output;
* proc print; * title 'Watermelon production 1993, Leesburg,Fl';

proc sort; by ID;
proc summary nway; class id; var weigh sugar;
     output out=yield mean=meanW meanS sum=sumaW sumaS;
     title 'Summary weight (W) sugar (S) watermelon/ production 1993, Leesburg,Fl';
* proc print;  /* complete printout is also requested below */

data GLM;
 set yield;
 if ID= 'prod' then delete;

/* assigning of infection time to each plant evaluated and harvested in the
field/plot3/1993 */
  inf_day=0;
  if ID='11-4' then inf_day='40';

 * if ID='11-14' or ID='5-12' then inf_day='54';

  if    ID='4-8'  or ID='5-13'  or ID='7-5'  or ID='9-6'  or ID='10-5' or ID='11-5'
     or ID='11-6' or ID='11-15' or ID='12-2' or ID='12-4' or ID='12-5' or ID='12-7'
     or   ID='12-14'
     then inf_day='57';

  if    ID='2-13' or ID='3-8'  or ID='3-12' or ID='4-7'   or ID='4-9'  or ID='4-12'
     or ID='5-11' or ID='6-9'  or ID='6-10' or ID='7-10'  or ID='9-2'  or ID='9-5'
     or ID='10-2' or ID='10-4' or ID='10-6' or ID='10-10' or ID='11-3' or ID='12-3'
     or ID='12-6' or ID='13-3' or ID='13-7' or ID='13-12' or ID='15-12'
     then inf_day='61';
```

```
if      ID='2-1'  or ID='2-20'  or ID='3-2'  or ID='3-10'  or ID='3-15'  or ID='4-10'
    or ID='4-14'  or ID='4-15'  or ID='5-5'  or ID='5-7'   or ID='5-8'   or ID='5-9'
    or ID='5-10'  or ID='5-15'  or ID='5-20' or ID='6-6'   or ID='6-11'  or ID='6-13'
    or ID='6-19'  or ID='7-9'   or ID='8-5'  or ID='8-8'   or ID='9-7'   or ID='9-10'
    or ID='9-15'  or ID='9-19'  or ID='10-8' or ID='10-13' or ID='10-15' or ID='10-16'
    or ID='10-17' or ID='10-20' or ID='11-1' or ID='11-2'  or ID='11-9'  or ID='11-19'
    or ID='12-14' or ID='12-15' or ID='13-1' or ID='13-2   or ID='13-5'  or ID='13-10'
    or ID='15-12'
    then inf_day='64';

if ID='1-4' or ID='1-5' or ID='1-6' or ID='1-8' or ID='1-14' or ID='1-15' or ID='1-18'
   or ID='1-20'or ID='2-3' or ID='2-7'  or ID='2-8' or ID='2-10' or ID='2-14'or ID='3-5'
   or ID='3-6' or ID='3-9' or ID='3-11' or ID='3-19'or ID='4-2'  or ID='4-5' or ID='4-11'
   or ID='4-13'or ID='4-16'or ID='4-17' or ID='4-19'or ID='4-20' or ID='5-1' or ID='5- 4'
   or ID='6-1' or ID='6-4' or ID='6-5'  or ID='6-7' or ID='6-12' or ID='6-14'
   or ID='6-17'or ID='6-20'or ID='7-1'  or ID='7-2' or ID='7-4'  or ID='7-12'
   or ID='7-19'or ID='10-1'or ID='11-12'
    then inf_day='68';

if ID='1-3' or ID='1-9' or ID='2-4' or ID='3-7' or ID='3-14' or ID='3-17' or ID='4-1'
    or ID='4-6'   or ID='5-6'   or ID='5-19'  or ID='6-2'   or ID='6-3'  or ID='6-8'
    or ID='8-2'   or ID='8-6'   or ID='8-13'  or ID='8-16'  or ID='8-17' or ID='9-3'
    or ID='9-8'   or ID='10-12' or ID='11-7'  or ID='11-10' or ID='11-11'or ID='11-13'
    or ID='11-18' or ID='11-20' or ID='13-17' or ID='13-19' or ID='14-2' or ID='14-8'
    or ID='14-15' or ID='14-18' or ID='15-5'  or ID='15-7'  or ID='15-8' or ID='15-18'
    or ID='15-20'
    then inf_day='71';


if ID='1-11' or ID='2-2' or ID='2-5' or ID='2-12' or ID='2-18' or ID='3-3'
   or ID='4-18' or ID='5-18' or ID='5-18'  or ID='6-16' or ID='6-18' or ID='7-6'
   or ID='7-8'  or ID='7-11' or ID='7-13'  or ID='7-16' or ID='7-20' or ID='8-3'
   or ID='8-4'  or ID='8-9'  or ID='8-10'  or ID='8-15' or ID='8-20' or ID='9-4'
   or ID='9-9'  or ID='9-13' or ID='9-14'  or ID='9-16'
   or ID='9-17' or ID='9-18' or ID='10-11' or ID='10-18' or ID='10-19' or ID='11-16'
   or ID='12-1' or ID='12-8' or ID='12-12' or ID='12-16' or ID='13-4' or ID='13-11'
   or ID='14-1' or ID='14-5' or ID='14-6' or ID='14-7' or ID='14-12' or ID='14-13'
   or ID='14-19' or ID='14-20' or ID='15-13'
   then inf_day='75';

if ID='1-1' or ID='1-10' or ID='1-13' or ID='1-19' or ID='2-6' or ID='2-9'
   or ID='2-11'  or ID='3-13'  or ID='3-20'  or ID='4-4'   or ID='5-2'   or ID='7-17'
   or ID='8-11'  or ID='8-19'  or ID='10-9'  or ID='11-17' or ID='12-11' or ID='12-18'
   or ID='13-9'  or ID='13-14' or ID='13-18' or ID='13-20' or ID='14-14' or ID='14-16'
   or ID='15-15' or ID='15-17' or ID='15-19'
   then inf_day='78';

if      ID='1-2'   or ID='1-17'  or ID='2-16'  or ID='3-1'  or ID='3-4'  or ID='4-3'
    or ID='5-3'   or ID='7-7'   or ID='8-18'  or ID='9-11' or ID='9-12' or ID='12-10'
    or ID='12-17' or ID='13-16' or ID='14-10' or ID='15-3' or ID='15-14'
    then inf_day='82';


 if ID='1-12' or ID='7-3' or ID='7-14' or ID='7-15' or ID='14-9' or ID='14-11'
    or ID='15-2' or ID='15-4'
    then inf_day='85';

 if ID='1-7' or ID='1-16' or ID='3-17' or ID='7-18' or ID='8-12' or ID='13-15'
    then inf_day='89';

 if ID='3-16' or ID='15-10'
    then inf_day='91';

 if Inf_day='0' then inf_day='.';


*-------------------------------------------------------------------------------*
| regression sum yield by each plant vs infection day                           |
|                                                                               |
*-------------------------------------------------------------------------------*;


  i2=(inf_day)*(inf_day);
  i3=i2*inf_day;
```

```
proc print;
   title 'Sum-yield by each plant and infection day after planting, Leesburg,Fl 1993';
run;

 proc glm; model sumaW=inf_day i2 i3;
   title 'GLM/summ-yield by each plant vs infection day after planting';

 proc glm; model sumaW=inf_day;
   title 'GLM/summ-yield by each plant vs infection day after planting';

 proc reg; model sumaW=inf_day/noint;
   title 'REG/summ-yield by each plant vs infection day after planting';

 proc reg; model meanW=inf_day/noint;
   title 'REG/mean-yield by each plant vs infection day after planting';

 proc reg; model meanW=inf_day;
   title 'REG/mean-yield by each plant vs infection day after planting';

 *-------------------------------------------------------------------------------*
 | regression sum-yield by all plants infected in the same day vs infection day  |
 |                                                                               |
 *-------------------------------------------------------------------------------*;

proc sort;by inf_day;
 title 'regression sum-yield by all plants infected in the same day vs infection day';

proc summary nway; class inf_day; var sumaW sumaS;  /* sum of all yield values by
plants/inf_day */
      output out=yield2 mean=meanWl meanSl sum=sumaWl sumaSl;
run;

data GLM;
set yield2;
   /* transformation of regression variables */
  inc=0;  /* to correct for incidence */
  if inf_day='40' then inc='0.0034';
  else if inf_day='54' then inc='0.0068';
  else if inf_day='57' then inc='0.0442';
  else if inf_day='61' then inc='0.0782';
  else if inf_day='64' then inc='0.146';
  else if inf_day='68' then inc='0.146';
  else if inf_day='71' then inc='0.132';
  else if inf_day='75' then inc='0.159';
  else if inf_day='78' then inc='0.098';
  else if inf_day='82' then inc='0.064';
  else if inf_day='85' then inc='0.023';
  else if inf_day='89' then inc='0.020';
  else if inf_day='91' then inc='0.0068';

  sumaWlc=sumaWl/inc; /* weigh incidence corrected */
  sumaSlc=sumaSl/inc; /* sugar incidence corrected */

  Wlog=log10(meanWl);
  ilog=log10(inf_day);
  i2=(inf_day)*(inf_day);
  i3=i2*inf_day;
  Wlogist=log10(meanWl/(52-meanWl));
  * Wmono=log10(52/(52-meanWl));  /* similar results than logistic transformation */
  * WGomp=LOG(52/LOG(52/meanWl)); /* similar results than logistic transformation */
proc print;
 title 'sum-yield by all plants infected in the same day after planting and transform
variables'; run;
   title 'regression sum-yield by all plants infected in the same day vs infection day';
proc glm; model sumawl=inf_day i2 i3;
proc glm; model sumawl=inf_day;
proc glm; model meanWl=inf_day;
proc glm; model sumaWlc=inf_day;
proc glm; model sumaWlc=inf_day i2 i3;
proc glm; model wlogist=inf_day;
proc glm; model sumaSlc=inf_day;
proc glm; model sumaSl=inf_day;
proc glm; model meanSl=inf_day;
      title 'GLM/of corrected and non-corrected weight and sugar content';
run;
```

```
proc reg; model sumaw1=inf_day/noint;
proc reg; model meanW1=inf_day/noint;
     title 'REG/of corrected and non-corrected weight and sugar content';
run;

* proc glm; * model wmono=inf_day; /* similar results than logistic transformation */
* proc glm; * model wgomp=inf_day; /* similar results than logistic transformation */
proc glm; model Wlog=ilog;
proc glm; model sumas1=sumaw1;
run;

proc corr spearman pearson;
  var inf_day meanS1 sumaS1 sumaS1c meanW1 sumaW1 sumaW1c Wlog ilog ;
  title 'Proc Corr/spearman pearson';
  run;
 proc plot; plot sumaW1*inf_day/vpos=15 hpos=30;
 proc plot; plot sumaW1c*inf_day/vpos=15 hpos=30;;
 proc plot; plot meanW1*inf_day/vpos=15 hpos=30;
 proc plot; plot sumaS1c*inf_day/vpos=15 hpos=30;;
 title 'Plot WEIGH vs infection day (after planting)';
 proc plot; plot sumaS1*sumaW1/vpos=15 hpos=30;
 title 'Sum sugar vs sum weigh, yield 1993';
 run;
```

Yield Loss Modeling: Data Set 1994

```
 *-------------------------------------------------------*
 | Watermelon yield 1994,  Data P3.  Leesburg,FL         |
 | first attempt  August 1,1994                          |
 | File:prod94\prod94_3.sas                              |
 *-------------------------------------------------------*;

 data prod94;
 infile 'a:\prod94\prod94-3.asc';
    input plot  section $  Fruit_No $  id $  Weigh1 sugar  SunDam $  BactDam $  HaHart $;
    Weigh=Weigh1*0.45359;       /* conversion of pounds to kilograms */
 output;
 * proc print; * title 'Watermelon production 1994, Data plot 3. Leesburg,Fl';
 run;

 proc sort; by ID;
 proc summary nway; class id; var weigh sugar;
     output out=yield mean=meanW meanS sum=sumaW sumaS;
     title 'Summary weigh (W) sugar (S) watermelon/ production 1994, Data plot 3
Leesburg,Fl';
 * proc print;  /* complete print out is also requested below */

 data GLM;
 set yield;

 if ID= 'prod' then delete;

  /* infection-time assigning to each plant evaluated and harvested in the
field/plot3/1994 */
  *-------------------------------------------------------------------------------------*;

 inf_day=0;


  if ID='5-12' or ID='6-13' or ID='12-18' or ID='13-2' or ID='15-18'
  then inf_day='48';  /* v-2-94  */


  if ID='1-8' or ID='2-14' or ID='5-14' or ID='11-2' or ID='11-17' or ID='12-6'
  then inf_day='51';


  if ID='3-19' or ID='4-15' or ID='5-11' or ID='5-13' or ID='6-5' or  ID='6-15'
  or ID='6-19' or ID='7-18' or ID='9-9'  or ID='9-20' or ID='10-9' or ID='11-6'
  or ID='13-3' or ID='13-14'
  then inf_day='54';
```

```
if ID='1-1' or ID='1-2' or ID='1-4' or ID='1-5' or ID='1-6' or ID='1-7' or ID='1-9'
or ID='1-10' or ID='1-11' or ID='1-12' or ID='1-14' or ID='1-15' or ID='1-16' or ID='1-17'
or ID='1-20' or ID='2-1'  or ID='2-3'  or ID='2-5'  or ID='2-6'  or ID='2-7' or ID='2-9'
or ID='2-10' or ID='2-11' or ID='2-12' or ID='2-13' or ID='2-16' or ID='2-17' orID='2-18'
or ID='2-20' or ID='3-1'  or ID='3-2'  or ID='3-3'  or ID='3-4'  or ID='3-6'
or ID='3-7'  or ID='3-11' or ID='3-14' or ID='3-15' or ID='3-16' or ID='3-17'
or ID='3-18' or ID='3-20' or ID='4-1'  or ID='4-5'  or ID='4-7'  or ID='4-8'  or ID='4-9'
or ID='4-10' or ID='4-11' or ID='4-12' or ID='4-14' or ID='4-16' or ID='4-18'
or ID='4-20' or ID='5-1'  or ID='5-2'  or ID='5-3'  or ID='5-5'  or ID='5-6'
or ID='5-15' or ID='5-16' or ID='5-17' or ID='5-19' or ID='6-1'  or ID='6-3'   or ID='6-4'
or ID='6-8'  or ID='6-10' or ID='6-11' or ID='6-12' or ID='6-14' or ID='6-16'
or ID='6-17' or ID='7-1'  or ID='7-3'  or ID='7-7'  or ID='7-11' or ID='7-13'
or ID='7-14' or ID='7-15' or ID='7-16' or ID='7-20' or ID='8-1'  or ID='8-2'  or ID='8-6'
or ID='8-7'  or ID='8-8'  or ID='8-11' or ID='8-14' or ID='8-15' or ID='8-19' or ID='8-20'
or ID='9-1'  or ID='9-5'
     or ID='9-10'  or ID='9-11'  or ID='9-14'   or ID='9-15'   or ID='9-16'  or ID='9-17'
     or ID='9-18'  or ID='10-1'  or ID='10-3'   or ID='10-4'   or ID='10-10' or ID='10-11'
     or ID='10-14' or ID='10-15' or ID='10-16'  or ID='10-18'  or ID='11-1'  or ID='11-5'
     or ID='11-10' or ID='11-12' or ID='11-13'  or ID='11-18'  or ID='11-19' or ID='11-20'
     or ID='12-1'  or ID='12-2'  or ID='12-7'   or ID='12-8'   or ID='12-12' or ID='12-15'
     or ID='12-16' or ID='12-17' or ID='12-19'  or ID='12-20'  or ID='13-1'  or ID='13-5'
     or ID='13-6'  or ID='13-8'  or ID='13-15'  or ID='13-16'  or ID='13-17' or ID='13-18'
     or ID='13-20' or ID='14-1'  or ID='14-2'   or ID='14-3'   or ID='14-4'  or ID='14-5'
     or ID='14-6'  or ID='14-7'  or ID='14-11'  or ID='14-13'  or ID='14-14' or ID='14-15'
     or ID='14-17' or ID='14-18' or ID='14-19'  or ID='14-20'  or ID='15-1'  or ID='15-7'
     or ID='15-10' or ID='15-11' or ID='15-14' or ID='15-16'   or ID='15-19' or ID='15-20'
 then inf_day='58';   /* v-12-94  */


if ID='1-3'  or ID='1-13' or ID='1-18' or ID='1-19' or ID='2-2'  or ID='2-4'  or ID='2-8'
 or D='2-13' or ID='2-15' or ID='3-5'  or ID='3-8'  or ID='3-9'  or ID='3-10' or ID='3-12'
 or ID='3-13' or ID='4-2'  or ID='4-4'  or ID='4-6'  or ID='4-13' or ID='4-17' or ID='5-4'
 or ID='5-8'  or ID='5-9'  or ID='5-10' or ID='5-18' or ID='5-20' or ID='6-2'  or ID='6-6'
 or ID='6-7'  or ID='6-9'  or ID='6-18' or ID='7-2'  or ID='7-5'  or ID='7-6'
 or ID='7-8'  or ID='7-9'  or ID='7-12' or ID='7-17' or ID='7-19' or ID='8-3'  or ID='8-4'
 or ID='8-5'  or ID='8-10' or ID='8-12' or ID='8-13' or ID='8-16' or ID='8-17'
 or ID='8-18'
     or ID='9-3'   or ID='9-8'    or ID='9-12'  or ID='9-19'  or ID='10-5'  or ID='10-6'
     or ID='10-17' or ID='10-19'  or ID='10-20' or ID='11-3'  or ID='11-7'  or ID='11-9'
     or ID='11-15' or ID='11-16'  or ID='12-3'  or ID='12-5'  or ID='12-10' or ID='12-14'
     or ID='13-4'  or ID='13-7'   or ID='13-9'  or ID='13-11' or ID='13-19' or ID='14-9'
     or ID='14-12' or ID='14-16'  or ID='15-2'  or ID='15-3'  or ID='15-6'  or ID='15-13'
     or ID='15-17'
 then inf_day='64';  /* evaluation date: V-18-94  */


 if ID='4-3' or ID='5-7' or ID='6-20' or ID='7-4' or ID='8-9' or ID='9-7' or ID='9-9'
     or ID='9-13'  or ID='10-7' or ID='10-8' or ID='10-12' or ID='11-4'  or ID='11-11'
     or ID='11-14' or ID='12-4' or ID='12-9' or ID='12-11' or ID='12-13'  or ID='13-10'
     or ID='15-5'  or ID='15-8' or ID='15-9' or ID='15-12' or ID='15-15'
 then inf_day='67';    /* evaluation date: v-21-94  */


if ID='7-10' or ID='9-2'   or ID='9-4'  or ID='9-6'   or ID='10-2' or ID='10-13'
or ID='11-8' or ID='13-13' or ID='14-8' or ID='14-10' or ID='15-4'
then inf_day='72';   /* evaluation date: v-26-94  */


if ID='13-12' then inf_day='79';   /* evaluation date: v1-2-94  */

if Inf_day='0' then inf_day='.';   /* only observation 12-19 missing  */


*-------------------------------------------------------------------------------*
| regression sum yield by each plant vs infection day                           |
|                                                                               |
*-------------------------------------------------------------------------------*;


  i2=(inf_day)*(inf_day);
  i3=i2*inf_day;

proc print;
   title 'Sum-yield by each plant and infection days after planting, Leesburg,FL 1994';
run;
```

```
 proc plot; plot sumaW *inf_day/vpos=15 hpos=30;
 proc plot; plot meanW *inf_day/vpos=15 hpos=30;
 proc plot; plot sumaS *inf_day/vpos=15 hpos=30;
 proc plot; plot sumaS *sumaW  /vpos=15 hpos=30;
      title 'Plot non-corrected Weight and Sugar vs infection day (after planting)';
 run;

 proc glm; model sumaW=inf_day i2 i3;
    title 'Cubic-GLM/sum-yield by each plant vs infection day after planting. Leesburg,FL
1994';
    run;

 proc glm; model sumaW=inf_day;
    title 'GLM/sum-yield by each plant vs infection day after planting.Leesburg,FL 1994';
    run;

 proc reg; model sumaW=inf_day/noint;
    title 'REG/sum-yield by each plant vs infection day after planting.Leesburg,FL 1994';
    run;

 proc glm; model meanW=inf_day;
    title 'GLM/mean-yield by each plant vs infection day after planting. Leesburg,FL
1994';
    run;

 proc reg; model meanW=inf_day/noint;
    title 'REG/mean-yield by each plant vs infection day after planting. Leesburg,FL
1994';
    run;



 *-------------------------------------------------------------------------------*
 | Data to regress sumw-yield by all plants infected in the same day vs infection day   |
 | Regression of sumw-yield by all plants infected in the same day vs infection day     |
 *-------------------------------------------------------------------------------*;

 proc sort;by inf_day;

 proc summary nway; class inf_day; var sumaW sumaS;  /* sum of all yield values by
plants/inf_day */
      output out=yield2 mean=meanW1 meanS1 sum=sumaW1 sumaS1;
 run;


 data GLM;
 set yield2;

   /* transformation of regression variables */

 inc=0;  /* to correct for incidence */

        if inf_day='48' then inc='0.0167';
    else if inf_day='51' then inc='0.0200';
    else if inf_day='54' then inc='0.0468';
    else if inf_day='58' then inc='0.5351';
    else if inf_day='64' then inc='0.2608';
    else if inf_day='67' then inc='0.0802';
    else if inf_day='72' then inc='0.0367';
    else if inf_day='79' then inc='0.0033';

 sumaW1c=sumaW1/inc; /* weigh incidence corrected */
 sumaS1c=sumaS1/inc; /* sugar incidence corrected */

 Wlog=log10(meanW1);
 ilog=log10(inf_day);
 i2=(inf_day)*(inf_day);
 i3=i2*inf_day;
 Wlogist=log10(meanW1/(52-meanW1));
   * Wmono=log10(52/(52-meanW1));  /* similar results than logistic transformation */
   * WGomp=LOG(52/LOG(52/meanW1)); /* similar results than logistic transformation */

 proc print;
      title 'sumw-yield by all plants infected in the same day and transformed variables';
 run;
```

```
proc glm; model sumaw1 =inf_day i2 i3;
proc glm; model sumaw1 =inf_day;
proc glm; model meanW1 =inf_day;
proc reg; model meanW1 =inf_day/noint;
proc glm; model sumaW1c=inf_day;
proc glm; model sumaW1c=inf_day i2 i3;
proc glm; model w1ogist=inf_day;
proc glm; model sumaS1c=inf_day;

* proc glm; * model wmono=inf_day; /* similar results than logistic transformation */
* proc glm; * model wgomp=inf_day; /* similar results than logistic transformation */

proc glm; model W1og  =ilog;
proc glm; model sumas1=sumaw1;
  title 'regression of sum-yield by all plants infected in the same day vs. time of
infection';
run;

proc corr spearman pearson;
     var inf_day meanS1 sumaS1 sumaS1c meanW1 sumaW1 sumaW1c W1og ilog ;
     title 'Proc Corr/spearman and pearson matrix';
run;

proc plot; plot sumaW1 *inf_day/vpos=15 hpos=30;
proc plot; plot sumaW1c*inf_day/vpos=15 hpos=30;
proc plot; plot meanW1 *inf_day/vpos=15 hpos=30;
     title 'Plot WEIGH vs infection day (after planting)';
proc plot; plot sumaS1c*inf_day/vpos=15 hpos=30;;
     title 'Fruit sugar content vs infection day (after planting)';
proc plot; plot sumaS1*sumaW1/vpos=15 hpos=30;
     title 'Sumn-sugar content vs. sumn-weigh, yield 1994';
run;
```

Nonstationary-Wave Dispersion model/Plot 1

```
*-------------------------------------------------------------------------------*
|SAS program to run the nonstationary wave dispersion model/Murray(1989)        |
| Eq.9.21, 238pp                                                                |
| Assumptions: time (t)=1 unit, Quantity (Insects released-Q-)=12                |
| Parameters to be estimated: m=shape of curve (see Okubo,1980 Fig 6.6,100p)     |
|                             n=size of the population (n-o-)                   |
|                             Do=Diffusion  coefficient -not uniform-           |
|                                                                               |
| Data source: Watermelon/plot 1, 1994, Leesburg, FL   data:D=Distance          |
|    INC=Incidence                                                              |
|                                                                               |
*-------------------------------------------------------------------------------*;

                        /* row sets 3, 2, 2, 2, 2,  last 2 eliminated */
                        /* Elimination of last point due to border efect */

data Disper;
input No ID $ D INC;
Q=15;
t=1;
if ID='plot1_2' then do; Q='28'; end;  /* _1=2May _2=5May _3=8May _4=12May */
else if ID='plot1_3' then do; Q= '37'; end;

cards;

                    1    plot1_1    46    13.3
                  3    plot1_1    52    12.5
                  4    plot1_1    58    12.5
                  5    plot1_1    64     7.5
                  6    plot1_1    70     2.5
                  7    plot1_1    76     2.5


                 10    plot1_2    46    20.0
```

```
11    plot1_2    52    27.5
12    plot1_2    58    27.5
13    plot1_2    64     7.5
14    plot1_2    70     2.5
15    plot1_2    76     5.0

17    plot1_3    46    30.0
19    plot1_3    52    40.0
20    plot1_3    58    37.5
21    plot1_3    64    12.5
22    plot1_3    70     5.0
23    plot1_3    76     7.5

PROC PRINT;
title 'Dispersion of ZYMV /WMV-2 by aphid vectors, D=dist(m) INC=inciden';
title2  'Q=est.  viruliferous  vect.  that  visited  the  viral  source  at  D=0,
evaluation time (t)=1 unit';
title3 'SAS program, April 1995';
run;

PROC SORT;BY ID;
PROC NLIN  BEST=10 MAXITER=70 G4SINGULAR METHOD=DUD;
     BY ID;
     parameters  m=1 to 5 by 0.15    n=1 to 12 by 1    Do= 0.01 to 1 by 0.01;

MODEL
INC=(n*(((t/((((((Q*GAMMA((1/m)+(3/2)))/((3.14**(1/2))*n*GAMMA((1/m)+1)))**2)*m
)/(2*Do*(m+2))))**(1/(2+m)))**-1))   *  ((1-(D/( (Q*GAMMA((1/m)+
(3/2)))/((3.14**(1/2))*n*GAMMA((1/m)+1)))* ((t/((((((Q*GAMMA((1/m)+
(3/2)))/((3.14**(1/2))*n*GAMMA((1/m)+1)))**2)*m)/ (2*Do*(m+2))))))**2)
**(1/m));      /* This is the SAS model from Eq.9.21 (Murray,1989) 258pp */

     output out=B  p=IncEST  r=IncRESID;
PROC SORT DATA=B;BY id;

PROC PRINT;
title2 'Wave dispersion model with PRON NLIN and DUD method and G4SINGULAR';
title3 'IncEST=incidence estimated by the model,INC=actual data, IncRESID=Inc-
IncEST';
run;

PROC PLOT  DATA=B; BY ID;
     PLOT INC*D= 'a'  IncEST*D='p'/OVERLAY VPOS=15;
     PLOT IncRESID*D/VREF=0 VPOS=15;
title2 'Plot of actual data (a), Predicted (p), and residuals vs distance';
title3;
run;
PROC GLM DATA=B; BY ID; MODEL IncEST=Inc;
TITLE2 'Regression to determine the goodness of fit for predicted data by the
wave dispersion model -nonstationary';
run;
```

Nonstationary-Wave Dispersion model/Plot 2

```
*-------------------------------------------------------------------------------*
| SAS program to run the nonstationary-wave dispersion model/Murray (1989)      |
| Eq. 9.21,238pp                                                                |
| Assumptions: time (t)=1 unit, Quantity (Insects released -Q- ) =12            |
| Parameters to be estimated: m=shape of curve (see Okubo,1980 Fig 6.6,100p)    |
| n=size of the population (n-o-)                                               |
| Do=Diffusion  coefficient -not uniform-                                       |
|                                                                               |
| Data source: Watermelon/plot 2, 1994, Leesburg, FL   Data:D=Distance          |
| INC=Incidence                                                                 |
*-------------------------------------------------------------------------------*;
```

```
            /* elimination of last three rows points due to border effect */
            /* m= smaller parameter value (0.5-4) in the model            */

Data Disper;
Input ID $ D INC;
Q=15;
t=1;
if ID='plot2_2' then do; Q='26'; end;  /* _1=2May _2=5May _3=8May _4=12May */
else if ID='plot2_3' then do; Q= '15'; end;
else if ID='plot2_4' then do; Q= '100'; end;
cards;

plot2_1 43  12.5
plot2_1 49  10.0
plot2_1 55  10
plot2_1 61  10
plot2_1 67   7.5
plot2_1 73   2.5

plot2_2 43  20
plot2_2 49  20
plot2_2 55  15
plot2_2 61  12.5
plot2_2 67  10
plot2_2 73  2.5

plot2_3 43  25
plot2_3 49  27.5
plot2_3 55  17.5
plot2_3 61  15
plot2_3 67  10
plot2_3 73  7.5

plot2_4 43  100
plot2_4 49  90
plot2_4 55  70
plot2_4 61  85
plot2_4 67  75
plot2_4 73  72.5

PROC PRINT;
title 'Dispersion of ZYMV/WMV-2 by aphid vectors, D=dist(m) INC=inciden';
title2  'Q=est. viruliferous vect. that visited the viral source at  D=0,
evaluation time (t)=1 unit';
title3 'SAS program, April 1995';
run;

PROC SORT;BY ID;
PROC NLIN  BEST=10 MAXITER=70 G4SINGULAR METHOD=DUD;
     BY ID;
      parameters  m=0.5 to 4 by 0.25     n=1 to 12 by 1     Do= 0.01 to 1 by
0.01;
MODEL
INC=(n*(((t/(((((Q*GAMMA((1/m)+(3/2)))/((3.14**(1/2))*n*GAMMA((1/m)+1)))**2)*m
)/(2*Do*(m+2))))**(1/(2+m)))**-1))*  ((1 (D/((Q*GAMMA((1/m)+ (3/2))) /
((3.14**(1/2)) * n * GAMMA((1/m)+1)))*((t/(((((Q*GAMMA((1/m)+
(3/2)))/((3.14**(1/2))*n*GAMMA((1/m)+1)))**2)*m) / (2*Do*(m+2)))))))**2)
**(1/m));      /* This is the SAS model from Eq.9.21 (Murray,1989) 258pp */

     output out=B  p=IncEST  r=IncRESID;
PROC SORT DATA=B;BY id;

PROC PRINT;
title2 'Wave dispersion model with PRON NLIN and DUD method and G4SINGULAR';
title3 'IncEST=incidence estimated by the model,INC=actual data, IncRESID=Inc-
IncEST';
run;
```

```
PROC PLOT  DATA=B; BY ID;
     PLOT INC*D= 'a'  IncEST*D='p'/OVERLAY VPOS=15;
     PLOT IncRESID*D/VREF=0 VPOS=15;
title2 'Plot of actual data (a), Predicted (p), and residuals vs distance';
title3;
run;

PROC GLM DATA=B; BY ID; MODEL IncEST=Inc;
TITLE2 'Regression to determine the goodness of fit of predicted data by
nonstationary-wave dispersion model';
run;
```

Disease gradients / Gregory and Exponential models

```
*-------------------------------------------------------------*
| SAS program to estimate disease gradient rates with         |
| the Gregory and exponential linearized models.              |
| Data 1994 (Plot 1 and 2). Leesburg,Fl.                      |
|  File name: in-spa94.sas.  Allocation: (:\Gregory\)         |
*-------------------------------------------------------------*;

data gradient;
input Site $  D Inc;
LND=log(d);
LNInc=log(inc);
cards;

plot1_1    46    13.3
plot1_1    52    12.5
plot1_1    58    12.5
plot1_1    64     7.5
plot1_1    70     2.5
plot1_1    76     2.5

plot1_2    46    20.0
plot1_2    52    27.5
plot1_2    58    27.5
plot1_2    64     7.5
plot1_2    70     2.5
plot1_2    76     5.0

plot1_3    46    30.0
plot1_3    52    40.0
plot1_3    58    37.5
plot1_3    64    12.5
plot1_3    70     5.0
plot1_3    76     7.5

plot2_1    43  12.5
plot2_1    49  10.0
plot2_1    55  10
plot2_1    61  10
plot2_1    67   7.5
plot2_1    73   2.5

plot2_2    43  20
plot2_2    49  20
plot2_2    55  15
plot2_2    61  12.5
plot2_2    67  10
plot2_2    73  2.5

plot2_3    43  25
plot2_3    49  27.5
plot2_3    55  17.5
plot2_3    61  15
plot2_3    67  10
plot2_3    73  7.5
```

```
plot2_4    43  100
plot2_4    49  90
plot2_4    55  70
plot2_4    61  85
plot2_4    67  75
plot2_4    73  72.5
;
proc print;
  title 'Disease gradients data /Watermelon 1994 Leesburg FL';
run;

/* Gregory model  */

proc sort; by site;
proc glm; by site;
  model LNInc=LND;
  output out=plotdat1 predicted=p;
  title 'Gregory model fitted to 1994 watermelon data/ Lesburg FL';
run;

proc sort; by site;
proc plot data=plotdat1; by site;
  plot p*LND='*' LNinc*LND='a'/overlay vpos=15 hpos=30;
  title 'LNInc and Predicted values with the Gregory model';
 run;

/* Exponential model  */

proc sort; by site;
proc glm; by site;
  model LNInc=D;
  output out=plotdat2 predicted=E;
  title 'Exponential model fitted to 1994 watermelon data / Lesburg,Fl';
run;

proc sort; by site;
proc plot data=plotdat2;by site;
  plot E*D='#' LNinc*D='T'/overlay vpos=15 hpos=30;
  title 'LNInc and Predicted values with the exponential model';
run;
```

Regression Analysis. Stage 1: Selection of Variables / Data sets 1993 and 1994

```
*------------------------------------------------------------*
| SAS program to select the most important aphid vectors     |
| as a preliminary stage for multiple regression analysis.   |
| Data 1993. Leesburg,Fl.  File name: inp2fac.sas.           |
| Varimax rotated biplots                                    |
*------------------------------------------------------------*;


data aphids93;
input days Ac Ag Am As Mp Af Up;
cards;
21          1     1     9     1     0     0     0
22          0     1     0     1     0     0     0
26          3     2     8     2     1     0     0
27          0     0     2     2     1     0     0
28          0     0     1     0     0     0     0
29          0     1     1     0     0     0     0
30          1     1     3     0     0     0     0
32          3     5    19     2     1     1     0
33          2     1     7     0     3     0     0
34          0     0     1     1     0     0     1
35          0     1     2     1     0     0     0
36          1     1     4     0     0     0     0
```

```
37          1     2     9     0     2     0     0
39          5     8     6     0     0     0     6
40          0     0     0     0     0     0     1
41          1    12     6     1     1     0    12
42          1    14     8     0     2     1     6
43          2     4     5     1     0     0     1
44          1    11    12     1     0     0    10
45          1     7     3     2     3     0     3
46          0     2     1     0     0     0     2
47          0     1     0     0     0     0     1
48          0     6     2     1     0     0     0
49          0     9     4     0     0     0    33
50          0     2     3     0     0     0    10
51          0     6     4     2     0     0    18
52          0     2     3     1     1     0    17
53          0     0     0     1     0     0     4
55          1     2     2     0     0     1     6
56          0     3     0     0     0     0     1
57          0     1     2     0     0     0     1
60          0     0     0     0     0     0     1
61          0     1     2     0     0     0     0
62          0     0     1     0     0     0     0
63          0     0     1     0     0     0     0
64          0     2     1     0     0     0     1
68          0     0     1     1     0     0     1
69          0     1     0     0     0     0     1
71          1     0     0     0     0     0     0
75          1     1     1     0     0     0     0
76          0     0     1     1     0     0     0
81          0     0     1     0     0     0     0
82          0     1     0     0     0     0     0
83          0     0     0     1     0     0     0
89          0     1     1     0     0     0     0
91          0     1     0     0     0     0     0
92          0     0     0     1     0     0     0
93          1     0     0     0     0     0     0
96          1     0     0     0     0     0     0
97          0     3     0     1     0     0     0
98          0     1     0     0     0     0     0
99          0     1     0     0     0     0     0
100         1     0     0     1     0     0     0
proc print;
 title 'Aphid vectors of WMV-2/ZYMV 1993, Leesburg, Florida';
run;

proc corr; var ac ag am as mp af up;
title 'Correlation matrix to identify potential high correlations';
run;

proc princomp out=pc prefix=prin;
 var Ac Ag Am As Mp Up; /* Af was eliminated to run similar data to 1994 */
 title 'First principal components analysis to preselect important variables';
run;

proc princomp out=pc prefix=prin;
 var Ag Am As Mp Up; /* Ac was eliminated because high loading in minor PC */
   title  'Second  principal  components  analysis  to  preselect  important
variables';
run;

proc factor method=principal rotate=varimax mineigen=1 plot preplot;
 var Ac Ag Am As Mp Up;
 title 'Varimax rotated biplots of eigenvalues > 1/all variables';
run;

proc factor method=principal rotate=varimax mineigen=1 plot preplot;
 var Ag Am As Mp Up;
 title 'Varimax rotated biplots of eigenvalues > 1/Ac eliminated';
run;

proc factor method=principal rotate=varimax mineigen=0.80 plot preplot;
```

```
 var Ac Ag Am As Mp Up;
 title 'Varimax rotated biplots of eigenvalues > 0.80 / All variables';
run;


*------------------------------------------------------------*
| SAS program to select the most important aphid vectors     |
| as a preliminary stage for multiple regression analysis.   |
| Data 1994. Leesburg,Fl.  File name: A:\in2fac94.sas.       |
| Varimax rotated biplots.                                   |
*------------------------------------------------------------*;


/* Preparatory analysis: Data infiled and listing */


OPTIONS LS=80 PS=60 PAGENO=1 NODATE CENTER;  /* 1. print options  */
DATA sample;                                 /* 2. name of data set */
   INFILE 'A:\in2fac94.dat';      /* 3. external file pca_expl.dat is input */
   INPUT days ag am mp up as ac;  /* 4. variables to be inputted data set */
OUTPUT;                           /* 5. output requested for the INFILE data*/
PROC PRINT;                       /* 6. the PRINTing procedure is requested */
     TITLE 'Print of pca_expl.dat data set';
                                  /* 7. a printout title for the data set */
RUN;                              /* 8.  the analyses procedure is executed */


/*  Step 1: Inspection of the correlation matrix and the structure of   */
/*          principal components                                        */

PROC CORR; VAR ac ag am as mp up;
    TITLE  'Correlation  matrix  to  identify  high  correl.  and  summary  of
variables';
  run;

PROC PRINCOMP DATA=sample;        /* 9.  the PROC PRINCOMP is specified  */
  VAR ag am mp up as ac;          /* 10. variable to be analyzed are listed */
  TITLE 'Principal component analysis of pca_expl.dat data set';
                                  /* 11. a title is defined for the PRINCOMP */
RUN;                              /* 12. the PRINCOMP procedure is executed */


/* Step 2 and 3: Inspection of the scree plot and major rotated principal */
/*               components                                               */


PROC FACTOR DATA=sample             /* 13. The FACTOR procedure is specified */
     METHOD=principal               /* 14. the method of PCA is invoked */
     NFACT=3 OUT=pca_out   /* 15. no. PCs to be rotated, name of new output */
     MINEIGEN=1             /* 16. specifies the cut-off value of the retained
PC (eigenv. > or = 1) */
     SCREE                  /* 17. the scree plot is requested */
     EIGENVECTORS           /* 18. nonrotated eigenvectors are inquired */
     ROTATE=varimax         /* 19. the varimax rotation method is specified */
     NPLOT=3 PLOT;    /* 20. three biplot display of mayor PC are requested */
* SIMPLE CORR;       /* 21. descriptive statistics and the corr. matrix */
  VAR ag am mp up as ac;   /* 22. variables to be analyzed are inputted  */
  TITLE 'Rotation of mayor PCs'; /* 23. a title is defined for SAS output */
RUN;                  /* 24. the FACTOR procedure is requested */

PROC PRINT DATA=pca_out;  /* 25. the PRINTing procedure of the new data set */
  VAR FACTOR1-FACTOR3;    /* 26. the scores of the PC 1 to 3 are requested  */
  TITLE 'PC scores of PCA_EXPLdata';  /* 27.printout title for new data */
  RUN;                                /* 28. the PRINT procedure executed */
```

Regression Analysis. Stage 2: Model Development Data Set 1993

```
                          *------------------------*
                          | LAG-FORECASTING 1993 |
                          *------------------------*;
 *-------------------------------------------------------------------------------*
 | Lag Forecasting 1993, first attempt. This program include several subprograms:  |
 | a) epidemics data transpose, b) total and average INC, c) Factor analysis,     |
 | d) merging data average INC and aphids                                         |
 | File: infolag1.sas.  Gainesville,FL March 17,1994                              |
 *-------------------------------------------------------------------------------*;

data waterm93;
   do plot=1 to 4;
      format pdate date7.;
      format idate date7.;
       if plot=1 then do;
         pdate='24mar93'd; idate='17may93'd; plants=294; max=14; end;
       else if plot=2 then do;
         pdate='24mar93'd; idate='24may93'd; plants=289; max=12; end;
       else if plot=3 then do;
         pdate='24mar93'd; idate='03may93'd; plants=294; max=16; end;
       else if plot=4 then do;
         pdate='24mar93'd; idate='17may93'd; plants=297; max=14; end;
    DO c=1 to max;
     INFILE 'A:\wm93sas\epid.dat';
      INPUT DATE DATE7. I;    /* I=number of new infected plants */
         FORMAT DATE DATE7.;
         DAY=DATE - IDATE;
         DAY_plan=DATE - PDATE;
         inc=I/plants;              /* Inc = % of I */
            plo=0;
             if plot='1' then do; plo='1';end;
             if plot='2' then do; plo='2';end;
             if plot='3' then do; plo='3';end;
             if plot='4' then do; plo='4';end;
         OUTPUT;
     END;
  END;

proc print; var plot day_plan Inc;   /* data listed in order of plots  */
TITLE1 'VIRUS EPIDEMICS ON WATERMELON, LEESBURG 1993';
run;
proc sort;by day_plan;
proc transpose out=test; by day_plan;
var inc;
id plo;
proc print;TITLE1 'VIRUS EPIDEMICS ON WATERMELON, LEESBURG 1993';
run;

 *-----------------------------------*
 | AVERAGE OF Inc FOR 4 PLOTS        |
 |                                   |
 *-----------------------------------*;

data average;
set test;
      if _1='.' then _1='0';
      if _2='.' then _2='0';
      if _3='.' then _3='0';
      if _4='.' then _4='0';
In4=(_1+_2+_3+_4);
inc4=(in4/4);
title1 'Average (INC4) and added (in4) Incidence of 4 watermelon plots, Leesburg 1993, _#
= plot number';
proc print;
run;

 *-----------------------------------*
 | Aphids spp/days after planting  |
 |                                   |
 *-----------------------------------*;
```

```
data aphids;
input  OBS  DAY_plan NAME $  AC   AG   AM   AS   MP   AF   UP;
 drop obs name;
  if AG = < '0' and day_plan > 20 then AG='0';
  if AM = < '0' and day_plan > 20 then AM='0';
  if UP = < '0' and day_plan > 20 then UP='0';
  if AC = < '0' and day_plan > 20 then AC='0';
  if MP = < '0' and day_plan > 20 then MP='0';
  if AS = < '0' and day_plan > 20 then AS='0';
  if AF = < '0' and day_plan > 20 then AF='0';

cards;
   1     21    COUNT      1    1    9    1    .    .    .
   2     22    COUNT      .    1    .    1    .    .    .
   3     26    COUNT      3    2    8    2    1    .    .
   4     27    COUNT      .    .    2    2    1    .    .
   5     28    COUNT      .    .    1    .    .    .    .
   6     29    COUNT      .    1    1    .    .    .    .
   7     30    COUNT      1    1    3    .    .    .    .
   8     32    COUNT      3    5   19    2    1    1    .
   9     33    COUNT      2    1    7    .    3    .    .
  10     34    COUNT      .    .    1    1    .    .    1
  11     35    COUNT      .    1    2    1    .    .    .
  12     36    COUNT      1    1    4    .    .    .    .
  13     37    COUNT      1    2    9    .    2    .    .
  14     39    COUNT      5    8    6    .    .    .    6
  15     40    COUNT      .    .    .    .    .    .    1
  16     41    COUNT      1   12    6    1    1    .   12
  17     42    COUNT      1   14    8    .    2    1    6
  18     43    COUNT      2    4    5    1    .    .    1
  19     44    COUNT      1   11   12    1    .    .   10
  20     45    COUNT      1    7    3    2    3    .    3
  21     46    COUNT      .    2    1    .    .    .    2
  22     47    COUNT      .    1    .    .    .    .    1
  23     48    COUNT      .    6    2    1    .    .    .
  24     49    COUNT      .    9    4    .    .    .   33
  25     50    COUNT      .    2    3    .    .    .   10
  26     51    COUNT      .    6    4    2    .    .   18
  27     52    COUNT      .    2    3    1    1    .   17
  28     53    COUNT      .    .    .    1    .    .    4
  29     55    COUNT      1    2    2    .    .    1    6
  30     56    COUNT      .    3    .    .    .    .    1
  31     57    COUNT      .    1    2    .    .    .    1
  32     60    COUNT      .    .    .    .    .    .    1
  33     61    COUNT      .    1    2    .    .    .    .
  34     62    COUNT      .    .    1    .    .    .    .
  35     63    COUNT      .    .    1    .    .    .    .
  36     64    COUNT      .    2    1    .    .    .    1
  37     68    COUNT      .    .    1    1    .    .    1
  38     69    COUNT      .    1    .    .    .    .    1
  39     71    COUNT      1    .    .    .    .    .    .
  40     75    COUNT      1    1    1    .    .    .    .
  41     76    COUNT      .    .    1    1    .    .    .
  42     81    COUNT      .    .    1    .    .    .    .
  43     82    COUNT      .    1    .    .    .    .    .
  44     83    COUNT      .    .    .    1    .    .    .
  45     89    COUNT      .    1    1    .    .    .    .
  46     91    COUNT      .    1    .    .    .    .    .
  47     92    COUNT      .    .    .    1    .    .    .
  48     93    COUNT      1    .    .    .    .    .    .
  49     96    COUNT      1    .    .    .    .    .    .
  50     97    COUNT      .    3    .    1    .    .    .
  51     98    COUNT      .    1    .    .    .    .    .
  52     99    COUNT      .    1    .    .    .    .    .
  53    100    COUNT      1    .    .    1    .    .    .
 ;

 proc factor  out=factors method=principal rotate=varimax nfactors=3;
   var AM AG UP;
 proc print;

title1 'Factor analysis with varimax rotation/scores of three most important factors
(AM,AG,UP)';
 run;
```

```
*------------------------------------*
| Merge Ind average and Aphids spp |
|                                    |
*------------------------------------*;

proc sort data=average;by day_plan;
proc sort data=aphids;by day_plan;
data merge;
merge average aphids;by day_plan;
 drop _name_;

 if AG = < '0' and day_plan > 20 then AG='0';
 if AM = < '0' and day_plan > 20 then AM='0';
 if UP = < '0' and day_plan > 20 then UP='0';
 if AC = < '0' and day_plan > 20 then AC='0';
 if MP = < '0' and day_plan > 20 then MP='0';
 if AS = < '0' and day_plan > 20 then AS='0';
 if AF = < '0' and day_plan > 20 then AF='0';

incX=inc4*30; /* scaling to plot with aphids in overlay mode */
 incS=in4*30;
 proc print;
title1 'Merge of aphids and acummulate (#) and average (*) incidence';

proc plot;
      plot IncX*day_plan='*' IncS*day_plan='#' AM*day_plan='M'  AG*day_plan='G'
      UP*day_plan='P' AC*day_plan='C'/overlay;
title 'Plot of WMV-2 incidence 1993';
run;



*-------------------------------------------------------*
| Multiple regression/lagged variables                  |
| Data set 1993 /with factor aphids scores              |
*-------------------------------------------------------*;


 data forcast2;
     set test;
     if _1='.' then _1='0';
     if _2='.' then _2='0';
     if _3='.' then _3='0';
     if _4='.' then _4='0';

     in4=(_1+_2+_3+_4);  /* acummulate incidence from 4 plots */
     proc sort;by day_plan;
     proc sort data=factors;by day_plan; /* these two sort is to prepare data for next
merging */


/* LAGGED VARIABLES WITH FACTORS SCORES TO FORECAST IN4 WITH APHID SPECIES */
/* ---------------------------------------------------------------------- */

data merge1;
  merge forcast2 factors;by day_plan;
  drop _1 _2 _3 _4 lin4_1 lin4_2 lin4_3 lin4_4 lin4_5;
  drop _name_ AM AG UP AC MP AF AS;
  if in4='.' and day_plan='81' then in4='0.616'; /*to move from 82 to 81 and avoid fac-
scrs missing */
* if in4='0.61676' then delete;  /* this should be done but for some reason it did not
work */


  inS= arsin (sqrt (in4));  /* in4 = add of 4 plots */

  /* LAGGED VARIABLES ESTIMATON */

* lfact1_0=lag15(factor1);     /* THESE LAGGED VAR (*) WERE NOT SIGNIFICANT IN PRELIMIAR
glm */
  lfact1_1=lag16(factor1);
* lfact1_2=lag17(factor1);
* lfact1_3=lag18(factor1);
  lfact1_4=lag19(factor1);
* lfact1_5=lag20(factor1);
```

```
                        /* THESE LAGGED VAR (*) WERE NOT SIGNIFICANT IN PRELIMIAR glm */
 * lfact2_0=lag34(factor2);
   lfact2_1=lag35(factor2);   /* lagg with respect the first peak */
 * lfact2_2=lag36(factor2);
 * lfact2_3=lag37(factor2);
 * lfact2_4=lag38(factor2);
 * lfact2_5=lag39(factor2);

 * lfact2_6=lag28(factor2);   /* lag with respect to the first peak AM (Factor 2) */
   lfact2_7=lag29(factor2);
   lfact2_8=lag30(factor2);
 * lfact2_9=lag31(factor2);
 * lfact2_A=lag32(factor2);
 * lfact2_B=lag33(factor2);

 * lfact3_0=lag25(factor3);   /* THESE LAGGED VAR (*) WERE NOT SIGNIFICANT IN PRELIMIAR
glm */
   lfact3_1=lag26(factor3);
   lfact3_2=lag27(factor3);
 * lfact3_3=lag28(factor3);
 * lfact3_4=lag29(factor3);
 * lfact3_5=lag30(factor3);


 proc print; title 'data forecasting/Factor scores of aphids/ day adjustment by aphids
peaks (about 30 days)';
  run;

 /* multiple regression without lagging variables */
 /* three factors included                        */
 /* ############################################## */

 proc reg data=mergel;
  model In4=Factor1 factor2 factor3/selection=stepwise;
  title 'data forecasting/non-lagged factor scores of aphids data';
  run;


 /* multiple regression with lagged variables    */
 /* iterative process of lag-variables selection */
 /* ############################################ */

/* PRELIMINARY GLM TO SELECT LAGGED VARIABLES OF FACTOR1 */
/* selection of variables by GLM with subgroups of variables and merging */
  proc glm; model in4= Lfact1_1  Lfact1_4/noint; * LFACT1_0  Lfact1_2; * Lfact1_3
LFACT1_5;
  title 'Data forecasting/GLM/factor1=UP, Final iteraction for selecting lagged-factor1
var';
  run;


/* PRELIMINARY GLM TO SELECT LAGGED VARIABLES OF FACTOR2 with respect highest peak */
  proc glm data=mergel; model in4= LFACT2_1/noint; * Lfact2_5 Lfact2_0; * Lfact2_1
Lfact2_2  Lfact2_3;
  title 'data forecasting/GLM/factor2=AM lst iteraction for selecting lagged-factor2 var';
  run;   /* note: none of these variables were significant/2_1 var corresponde with peak
*/


/* PRELIMINARY GLM TO SELECT LAGGED VARIABLES OF FACTOR2 with respect first peak */
  proc glm data=mergel; model in4=Lfact2_7 Lfact2_8/NOINT; *LFACT2_6 Lfact2_9 Lfact2_A
Lfact2_B;
  title 'data forecasting/GLM/factor2=AM 2nd iteraction for selecting lagged-factor2 var';
  run;    /* note: none of these variables were significant/ 2_8 corresponde with peak */


/* PRELIMINARY GLM TO SELECT LAGGED VARIABLES OF FACTOR3 */
  proc glm data=mergel; model in4= Lfact3_2 Lfact3_1/noint; * Lfact3_0  Lfact3_5; *
Lfact3_3 LFACT3_4;
  title 'data forecasting/GLM/factor3=AG, Final iteraction for selecting lagged-factor3
var';
  run;
```

```
/* multiple regression with lagged variable from factors 1-3 */

/* ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++ */


  proc reg data=merge1;    /* subgroup 1 */
  model In4=Lfact1_1 lfact3_2  Lfact2_8/noint selection=stepwise;
  title 'data forecasting/lagg of three factor scores,subgroup 1 vs. accumulate incidence
(in4)';
  run;

  proc reg data=merge1;   /* subgroup 2 */
  model In4= Lfact1_4 lfact3_1  Lfact2_1  /noint selection=stepwise;
  title 'data forecasting/lagg of three factor scores,subgroup 2 vs. accumulate incidence
(in4)';
  run;

  proc reg data=merge1;   /* merging variables selected from subgroups 1 and 2 */
  model In4=Lfact1_1 Lfact1_4 Lfact3_2  Lfact2_8/noint selection=stepwise;
  output out=plotdat p=est;
  title 'data forecasting/lagg of three factor scores,merging groups 1 and 2 vs. acumm inc
(in4)';
  proc plot data=plotdat; plot in4*day_plan='a' est*day_plan='p'/overlay;
  proc print data=plotdat; var day_plan in4 est;
  run;

  proc reg data=merge1;   /* merging variables selected from subgroups 1 and 2 */
  model In5=Lfact1_1 Lfact1_4 Lfact3_2  Lfact2_8/noint selection=stepwise;
  title 'data forecasting/lagg of three factor scores,merging groups 1 and 2 vs. arsin inc
(in4)';
  run;


  *-------------------------------------------------------------------------*
  |other combination of variables tested:                                   |
  | 1_1 2_1 3_1 and 1_4 2_8 final selection 1_4 r2=0.41                     |
  | 1_1 2_1 1_4 and 3_1 2_8 final           1_4 r2=0.50                     |
  | 1_1 2_1 2_8 2_7 and 1_4 3_1 and 3_2 final 1_1 1_4 2_7  subgroups A and B |
  | regression for these last group shown below                             |
  *-------------------------------------------------------------------------*;

  proc reg data=merge1;   /* merging variables selected from subgroups A and B */
  model In4=Lfact1_1 Lfact1_4  Lfact2_1 lfact2_7 /noint selection=stepwise;
  title 'Forecasting/lagg of three factor scores,merging groups A and B (not shown) vs.
acumm inc (in4)';
   run;
```

Regression Analysis. Stage 2: Model Development Data Set 1994

```
                              *----------------------*
                              | LAG-FORECASTING 1994 |
                              *----------------------*;
 *-------------------------------------------------------------------------------*
 | Lag Forecasting 1994, first attempt. This program include several subprograms:  |
 | a) epidemics data transpose, b) total and average INC, c) Factor analysis,     |
 | d) merging data average INC and aphids                                         |
 | File: inllag94.sas (infolag1.sas in 1993 data). Gainesville,FL June 29,1994    |
 *-------------------------------------------------------------------------------*;
 * ;
 * ;
 * ;

 *-------------------------------------------------------------------*
 | Epidemics data transpose. Data 1994 ordered by days after         |
 | planting.                                                         |
 | Objective:to generate a output to calculate the average incidence |
 *-------------------------------------------------------------------*;

data waterm94;
   do plot=1 to 4;
      format pdate date7.;
```

```
      format idate date7.;
       if plot=1 then do;
         pdate='15mar94'd; idate='25Apr94'd; plants=298; max=9; end;
/* idate=infection date */
       else if plot=2 then do;
         pdate='15mar94'd; idate='25Apr94'd; plants=299; max=9; end;
       else if plot=3 then do;
         pdate='15mar94'd; idate='02may94'd; plants=299; max=9; end;
       else if plot=4 then do;
         pdate='15mar94'd; idate='28Apr94'd; plants=299; max=11; end;
    DO c=1 to max;
     INFILE 'A:\wm94sas\epid.dat';
      INPUT DATE DATE7. I;    /* I=number of new infected plants */
         FORMAT DATE DATE7.;
         DAY=DATE - IDATE;
         DAY_plan=DATE - PDATE;
         inc=I/plants;              /* Inc = % of I */
         plo=0;
             if plot='1' then do; plo='1';end;
             if plot='2' then do; plo='2';end;
             if plot='3' then do; plo='3';end;
             if plot='4' then do; plo='4';end;
         OUTPUT;
    END;
  END;
proc print; var plot day_plan Inc;   /* data listed in order of plots  */
TITLE1 'VIRUS EPIDEMICS IN 4 WATERMELON PLOTS. INCIDENCE BY PLOTS. LEESBURG, FL 1994';
run;
proc sort;by day_plan;
proc transpose out=test; by day_plan;
var inc;
id plo;
proc print;
TITLE1 'VIRUS EPIDEMICS ON 4 WATERMELON PLOTS.INCIDENCE BY DAYS AFTER PLANTING LEESBURG,FL
1994';
run;
 *-------------------------------------------------------*
 | Total and average incidence for 4 plots. Data 1994 |
 | objective: to determine the dependent variable     |
 *-------------------------------------------------------*;

data average;
set test;
      if _1='.' then _1='0';
      if _2='.' then _2='0';
      if _3='.' then _3='0';
      if _4='.' then _4='0';

In4=(_1+_2+_3+_4);
inc4=(in4/4);
title1 'Average (INC4) and total (in4) virus incidence of 4 watermelon plots, _# = plot
number';
proc print;
run;


 *-------------------------------------------------------------------------------------*
 | Aphids94 spp/days after planting. Proc factor analysis after inspecting minor-pc    |
 | and biplots (see file:INPPAC94.SAS). In this second analysis, AC was not included.  |
 | the output used is the factor scores.  File:inllag94.sas (in 1993 data:infolag1.sas |
 | objective: to determine the independent variables.                                  |
 *-------------------------------------------------------------------------------------*;

data aphids;
input  OBS  DAY_plan  AG   AM   MP   UP    AS   AC;
 drop obs;
  /* if AG = < '0' and day_plan > 20 then AG='0';  */
  /* if AM = < '0' and day_plan > 20 then AM='0';  */
  /* if UP = < '0' and day_plan > 20 then UP='0';  */
  /* if AC = < '0' and day_plan > 20 then AC='0';  */
  /* if MP = < '0' and day_plan > 20 then MP='0';  */
  /* if AS = < '0' and day_plan > 20 then AS='0';  */
  /* if AF = < '0' and day_plan > 20 then AF='0';  */

/* *if-and-then* not needed since aphids data was already zeros-corrected:APHIDS94.prn */
```

```
cards;
```

1	8	1	1	0	0	0	0
2	9	1	3	6	0	0	0
3	10	1	1	2	0	0	0
4	11	2	4	0	2	0	0
5	13	0	2	3	0	1	0
6	14	0	0	1	0	0	0
7	15	2	2	1	2	2	0
8	16	1	0	6	1	0	1
9	17	0	2	1	4	0	0
10	18	4	5	1	8	0	0
11	20	3	4	0	1	0	1
12	21	0	2	0	7	0	0
13	22	4	2	2	3	0	1
14	23	0	0	2	6	0	0
15	24	1	3	16	3	6	0
16	25	0	3	4	1	0	0
17	27	1	1	0	3	1	0
18	28	0	1	0	0	0	0
19	29	4	4	2	8	0	0
20	30	1	0	0	121	1	1
21	31	2	3	1	311	0	0
22	32	1	0	0	19	1	0
23	34	0	4	14	35	5	0
24	35	0	6	11	6	0	0
25	36	2	9	1	45	0	0
26	37	1	1	0	15	0	1
27	38	2	2	0	71	1	0
28	39	0	0	0	43	1	0
29	41	0	4	33	15	0	0
30	42	0	3	2	13	0	0
31	43	1	3	2	17	1	0
32	44	0	0	1	6	0	0
33	45	0	3	0	8	0	0
34	47	0	0	0	158	3	0
35	48	1	1	0	31	3	0
36	49	1	0	0	8	0	0
37	50	1	0	0	319	0	1
38	51	2	0	0	42	0	0
39	52	1	3	1	20	0	0
40	54	1	2	1	23	2	0
41	55	0	0	0	1	1	0
42	56	1	1	0	6	1	0
43	57	0	1	0	5	0	1
44	58	0	1	0	1	0	0
45	59	1	0	0	8	0	0
46	61	1	0	0	3	1	1
47	62	0	1	0	0	0	1
48	63	0	0	0	1	1	0
49	68	0	1	0	1	0	0
50	69	0	1	0	0	0	0
51	72	0	0	0	0	1	0
52	75	0	1	0	0	1	0
53	77	0	1	0	0	0	2
54	78	0	0	0	0	1	0
55	79	0	1	0	0	1	0
56	80	0	0	0	1	0	1
57	84	0	0	0	0	2	0
58	85	1	0	0	0	1	0
59	86	0	0	0	0	1	0
60	87	0	0	0	0	2	0
61	90	1	1	0	0	3	0
62	91	1	0	0	0	0	0
63	92	0	0	0	0	2	0
64	93	0	0	0	1	2	0

```
proc corr; var AG MP UP AS AM;
    /* by observing the proc corr output AM was corr with AG. */
proc print;
title 'Correlation Matrix of five spp of aphids. Data 1994';
run;

proc factor  out=factors method=principal rotate=varimax nfactors=4;
 * var AG AM MP UP AS;
```

```
   /* by observing the output of the previous statement AM was eliminated  */
   /* by observing the proc corr above AM was corr with AG. */
   /* I decide to test AM and elim. AG(this factor was never high corr. with In4 (0.40) */

  *var AG MP UP AS;/* this statement is requested to leave only orthogonal matrix and AG
*/
     /* with previous statement: Fac1=UP (lag7),F2=MP(lag15),F3=AG (lag25),F4=AS(lag22) */
     /* maximun r-square found 0.79 with UP lagged at 7 time units                      */
  var AM MP UP AS; /* this statement is requested to leave only orthogonal matrix and AM
*/
     /* with previous statement: Fac1=AS (Lagg21,29) F2=AM (19,20,34), F3=UP (7,10)     */

proc print;
title1 'Factor analysis-varimax rotation/scores of most important factors(aphid  spp)';
run;


*-------------------------------------------------*
| Merge data sets: AVERAGE and APHIDS             |
| objective: Ploting Incidence vs. aphids spp     |
*-------------------------------------------------*;

proc sort data=average;by day_plan;
proc sort data=aphids;by day_plan;
data merge;
merge average aphids;by day_plan;
 drop _name_;
 UP_s=UP/10;          /* scaling UP for ploting with other aphids    */
 AM_s=AM*3;
 AG_s=AG*5;
 incX=inc4*20;        /* scaling to plot with aphids in overlay mode */
 incS=in4*20;
 proc print;
title1 'Merging of aphid data and total(INC4) and average(IN4) and scaled(_XS) incidence';
proc plot;
plot IncX*day_plan='*'  IncS*day_plan='#'  MP*day_plan='P'  AM_s*day_plan='M'
      AG_s*day_plan='G'    UP_s*day_plan='U' AS*day_plan='S'/overlay;
title 'Plot of aphid data and total (#) and average (*) scaled incidence';
run;


*-----------------------------------------------------------*
|Merging data sets: AVERAGE and FACTORS  and definition of  |
|lagged factor scores. Original aphids counts are droped    |
|objective: to define the forecasting data matrix           |
*-----------------------------------------------------------*;


 data premerge;
    set average;
    proc sort data=average;by day_plan;
    proc sort data=factors;by day_plan;
      /* these two proc sort are done to prepare data for next merging   */

data merge1;
    merge average factors;by day_plan;
    drop _1 _2 _3 _4;
      /* these are the proportion of incidence change by individual plots */
    drop _name_ AM AG UP AC MP AS;
      /* these are the original aphid counts                              */

  * if day_plan=54 then do; *  in4='.' ; * end;
  * else if day_plan=55 then do; * in4='0.160'; * end;

     /*to move from 54 to 55 and avoid factor scores missing at this important peak */


  inS= arsin (sqrt (in4));  /* in4 = total proportion of incidence change of 4 plots */
     /* this transformation is because the incidence data has a proportion units    */


/* Estimation of lagged variables for factor scores to be used as independent var */
/* ------------------------------------------------------------------------------ */
```

```
 *  lfact1_0=lag20(factor1); /* THESE LAGGED VAR (*)WERE NOT SIGNIF. IN PRELIMIAR glm */
    lfact1_1=lag21(factor1);
 *  lfact1_2=lag22(factor1); /*  factor1= AS  */
 *  lfact1_3=lag28(factor1);
    lfact1_4=lag29(factor1);
 *  lfact1_5=lag30(factor1);
 *  lfact1_6=lag31(factor1);

                             /* THESE LAGGED VAR(*) WERE NOT SIGNIF. IN PRELIMIAR glm */
 *  lfact2_0=lag21(factor2);
    lfact2_1=lag20(factor2);  /* lagg with respect the first peak AM */
    lfact2_2=lag33(factor2);
 *  lfact2_3=lag18(factor2);
 *  lfact2_4=lag17(factor2);
 *  lfact2_5=lag16(factor2);


 *  lfact2_6=lag35(factor2); /* lag with respect to secondary first peak AM (Factor 2) */
    lfact2_7=lag34(factor2);
    lfact2_8=lag19(factor2);
 *  lfact2_9=lag32(factor2);
 *  lfact2_A=lag31(factor2);
 *  lfact2_B=lag30(factor2);

*  lfact3_0=lag6(factor3);  /* THESE LAGGED VAR (*) WERE NOT SIGNF. IN PRELIMIAR glm */
    lfact3_1=lag7(factor3);
    lfact3_2=lag10(factor3); /* Factor3=UP  */
 *  lfact3_3=lag9(factor3);
 *  lfact3_4=lag31(factor3);
 *  lfact3_5=lag32(factor3);


 *  lfact4_0=lag15(Factor4);  /*  this set was used for data set  AG AS MP UP  */
 *  lfact4_1=lag16(Factor4);
 *  lfact4_3=lag21(Factor4);
 *  lfact4_4=lag22(Factor4);

 *  MPAS=lfact2_7 * Lfact4_0; /* this work for data set AG AS UP MP, R-SQUARE 0.80 */
    ASAM=lfact1_1 * Lfact2_8;
    ASAM1=Lfact1_4 * Lfact2_7;

proc print;
title 'Data forecasting/Factors and Factor scores of aphids lagged by time';
run;


/* multiple regression without lagging variables */
/* three factors included                        */
/* ############################################# */

 proc reg data=mergel;
      model In4=Factor1 factor2 factor3 Factor4/selection=stepwise;
 title 'data forecasting/non-lagged factor scores of aphids data';
 run;


/* multiple regression with lagged variables    */
/* iterative process of lag-variables selection */
/* ############################################ */


/* PRELIMINARY GLM TO SELECT LAGGED VARIABLES OF FACTOR1 */
/* selection of variables by GLM with subgroups of variables and merging */

/* proc glm; model in4=  Lfactl_0  Lfactl_2 Lfactl_3  Lfactl_5/noint;    */
   proc glm; model in4=  LFACT1_1 Lfactl_4 /noint;
   title 'Data forecasting/GLM/factor1=AS,Final iteraction for select. lagged-fact.1 var';
   run;            /* note: Lfactl_1 significant                         */


/* PRELIMINARY GLM TO SELECT LAGGED VARIABLES OF FACTOR2 */

   proc glm data=mergel; model in4= Lfact2_1 Lfact2_2/noint;
/* proc glm data=mergel; model in4= LFACT2_0 LFACT2_3 Lfact2_4 Lfact2_5/noint;         */
```

```
    title 'data forecasting/GLM/factor2=AM 1st iteraction for selecting lagged-factor2
var';
   run;   /* note: 2_1 2_2 were significant/2_1 var correspond with peak                */
/* PRELIMINARY GLM TO SELECT LAGGED VARIABLES OF FACTOR2 # some runned and deactivated
*/

   proc glm data=mergel; model in4=Lfact2_7 Lfact2_8/NOINT;
/* proc glm data=mergel; model in4= LFACT2_6 Lfact2_9 Lfact2_A  Lfact2_B /NOINT;       */
    title 'data forecasting/GLM/factor2=AM 2nd iteraction for selecting lagged-factor2
var';
   run;  /* note: 2_7 2_8  were significant/ 2_8 correspond with peak                  */


/* PRELIMINARY GLM TO SELECT LAGGED VARIABLES OF FACTOR3 # some runned and deactivated
*/

   proc glm data=mergel; model in4= Lfact3_2 Lfact3_1/noint;
/* proc glm data=mergel; model in4= Lfact3_0  Lfact3_3 Lfact3_4 LFACT3_5/noint;       */
   title 'data forecasting/GLM/factor3=UP,Final iteraction for selecting lagged-fac3 var';
   run;  /* note: 3_1 3_2  were significant/ 3_1 correspond with highest peak          */


/* PRELIMINARY GLM TO SELECT LAGGED VARIABLES OF FACTOR4/runned only with AG UP AS MP
*/
/* GLM's runned and deactivated */

  /*  proc glm data=mergel; model in4= Lfact4_0 Lfact4_1 /noint;                       */
  /*  title 'GLM/factor4=AS, Final iteraction for selecting lagged-factor3 var';       */
  /*  run;                                                                            */


/* multiple regression with lagged variable from factors 1-3 */
/* ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++ */


  proc reg data=mergel;    /* subgroup 1 */
       model In4=Lfactl_1 Lfact2_1 lfact2_8  Lfact3_1  ASAM/ selection=stepwise;
       title 'Lagg of three factor scores,subgroup 1 vs. accumulate incidence (in4)';
  run;

  proc reg data=mergel;   /* subgroup 2 */
  model In4= Lfactl_1  Lfact2_2  lfact2_7  Lfact3_2  ASAM/noint selection=stepwise;
      title 'Lagg of three factor scores,subgroup 2 vs. accumulate incidence (in4)';
      run;

  proc reg data=mergel;   /* merging variables selected from subgroups 1 and 2 */
  model In4=Lfact3_1  Lfact3_2 ASAM /noint selection=stepwise;
  output out=plotdat p=est;
       title 'Lagg of three factor scores,merging groups 1 and 2 vs. acumm inc (in4)';
  proc plot data=plotdat; plot in4*day_plan='a' est*day_plan='p'/overlay;
  proc print data=plotdat; var day_plan in4 est;
      run;

  proc reg data=mergel;   /* merging variables selected from subgroups 1 and 2 */
  model InS=Lfactl_1 Lfact3_2 Lfact3_1 Lfact2_7 Lfact2_8/noint selection=stepwise;
  title 'data forecasting/lagg of five variables scores  vs. arsin inc (in4)';
  run;

  proc reg data=mergel;   /* merging variables selected from subgroups 1 and 2 */
  model inS=Lfact3_2 Lfact3_1 ASAM/noint selection=stepwise;
  title 'data forecasting/lagg of three variables scores 2 vs. arsin inc (in4)';
  run;

  proc reg data=mergel;   /* merging variables selected from subgroups 1 and 2 */
  model inc4=Lfact1_2 Lfact3_1 ASAM/noint selection=stepwise;
  title 'data forecasting/lagg of three variables scores 2 vs. arsin inc (in4)';
  run;

  *-------------------------------------------------------------------------------*
  |other combination of variables tested:                                         |
  *-------------------------------------------------------------------------------*/
  proc glm data=mergel;   /* merging variables selected from subgroups A and B */
  model In4=Lfactl_1 Lfact2_7 Lfact2_8 Lfact3_1 Lfact3_2 /noint ; * selection=stepwise;
  title 'Forecasting/lagg of all variables vs. acumm inc (in4)'; run;
```

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BIOGRAPHICAL SKETCH

Gustavo Mora Aguilera was born on November 26, 1962, in Apatzingán Michoacán, México. Gustavo attended the Universidad Autónoma Chapingo in Chapingo, México, between 1980 and 1985, where he earned a Bachelor of Science degree of Ingeniero Agrónomo with a major in Parasitología Agrícola. In 1985, he assumed the position of research assistant in the section of fruit crop diseases at the Department of Plant Pathology, Colegio de Postgraduados, Montecillo, México. From 1989 to 1990, Gustavo completed his Master of Science degree in plant pathology at the same institution. As training in plant disease epidemiology, Gustavo expended the fall of 1990 and the spring of 1991 at North Carolina State University, and beginning in 1992 initiated studies at the University of Florida to pursue a doctoral degree in plant pathology. Upon completion of his studies, Gustavo will rejoin the Colegio de Postgraduados, where he will collaborate in research programs and teaching in crop epidemiology and integrated pest management.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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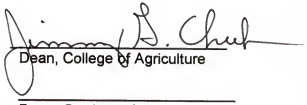

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